UNIVERSITY OF LONDON THESIS

Degree PhD Year 2006 Name of Author FADEN Asma Ahmad

COPYRIGHT

This is a thesis accepted for a Higher Degree of the University of London. It is an unpublished typescript and the copyright is held by the author. All persons consulting the thesis must read and abide by the Copyright Declaration below.

COPYRIGHT DECLARATION

I recognise that the copyright of the above-described thesis rests with the author and that no quotation from it or information derived from it may be published without the prior written consent of the author.

LOAN

Theses may not be lent to individuals, but the University Library may lend a copy to approved libraries within the United Kingdom, for consultation solely on the premises of those libraries. Application should be made to: The Theses Section, University of London Library, Senate House, Malet Street, London WC1E 7HU.

REPRODUCTION

University of London theses may not be reproduced without explicit written permission from the University of London Library. Enquiries should be addressed to the Theses Section of the Library. Regulations concerning reproduction vary according to the date of acceptance of the thesis and are listed below as guidelines.

A. Before 1962. Permission granted only upon the prior written consent of the author. (The University Library will provide addresses where possible).

B. 1962 - 1974. In many cases the author has agreed to permit copying upon completion of a Copyright Declaration.

C. 1975 - 1988. Most theses may be copied upon completion of a Copyright Declaration.

D. 1989 onwards. Most theses may be copied.

This thesis comes within category D.

☐ This copy has been deposited in the Library of UCL

☐ This copy has been deposited in the University of London Library, Senate House, Malet Street, London WC1E 7HU.

Viruses associated with potentially malignant and malignant oral mucosal lesions

Thesis submitted by

Asmaa A. Faden BDS, MDS

for the degree of

DOCTOR OF PHILOSOPHY

in the

Faculty of Medicine

University of London

Department of Oral Medicine

Division of Microbial Diseases

Eastman Dental Institute

University College of London

256 Gray's Inn Road

London

WC1X 8LD

UK

2006

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

Abstract

Oral squamous cell carcinoma (OSCC) and oral epithelial dysplasia (OED) are known to be associated with high tobacco and/or alcohol consumption. A viral aetiology for OSCC and OED has been proposed, in particular human papillomavirus (HPV).

The overall aim of the present work was to determine the geographical prevalence and viral interactions of HPV, CMV, EBV and HHV-8 in oral squamous cell carcinoma (OSCC) and potentially malignant oral mucosal diseases (oral epithelial dysplasia (OED), oral lichen planus (OLP) and proliferative verrucous leukoplakia (PVL)), as well as a range of salivary gland tumours and samples from HIV infected individuals.

Extraction of DNA from archival paraffin-embedded lesional tissues of histopathologically proven OSCC, OED, OLP, PVL, and normal oral mucosa of HIV-infected individuals, various salivary gland tumours and normal oral mucosa was performed. Viral DNA of HPV, human herpesvirus (HHV-8), Epstein-Barr virus (EBV) and cytomegalovirus (CMV) was detected by nested polymerase chain reaction (PCR), and the genotype of HPV was determined using reverse line blotting hybridisation (RLB).

The proportion of HPV-DNA detected in OSCC was higher when compared to all other examined mucosal and salivary gland lesions. There were trends of an increased prevalence of HPV within OED, PVL and OLP when compared to normal-control oral mucosa. The proportion of HHV-8 was not increased in any oral mucosal lesion except in HIV-individuals when compared to normal-control, which was expected. With respect to EBV, the

proportion of EBV in PVL samples was increased when compared to normal-control. The CMV-DNA was detected in a low percentage of both PVL and OED samples when compared to the normal-control. There was no evidence of any interactions between any herpes viruses with HPV in OSCC.

It is concluded that HPV infection may be associated with OSCC and OED.

Acknowledgements

I would like to express my appreciation and gratitude to my supervisor Professor Stephen Porter for his constant advice, understanding, and patience throughout my PhD journey. I am particularly grateful for the help of my co-supervisor Dr David Moles with the statistical analysis of this research. I would also like to thank Dr Chong Gee Teo who facilitated my research at the Health Protection Agency in Colindale.

I am particularly grateful to my family, especially my husband Rushdi Felimban for his unfailing support and belief in me, which has helped me achieve my goals. Special thanks to the gems of my life, my three little children (Ghada, Omar and Tarik), hoping that they will cherish and appreciate my achievements.

Many thanks go to my colleagues at the Health Protection Agency (HPA), Colindale, London and the Eastman Dental Institute-UCL, especially Miss Nichola King for her friendship and encouragement.

I would like to dedicate this thesis to my late parents and special thanks to my brother Fahad and my sister Etidal and to the rest of my family.

Abbreviations

bp	base pair
℃	degree celsius
CC	cervical cancer
CdK	cyclin dependent kinase
CIN	cervical intraepithelial neoplasia
CMV	cytomegalovirus
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
EBV	Epstein-Barr virus
EDTA	ethylenediaminetetra-acetate
h	hour
HAART	highly active antiretroviral therapy
HHV-8	human herpesvirus-8
HHV	human herpesviruses
HIV	human immunodeficiency virus
HNSCC	head and neck squamous cell carcinoma
HPV	human papillomavirus
HSIL	high-grade squamous intraepithelial lesions
ICC	intracervical cancer
ISH	*in situ* hybridization
kb	kilobase
KS	Kaposi's sarcoma

L	litre
LOH	loss of hetrozygosity
m	minute
mg	milligram
µl	microlitre
ml	millilitre
mRNA	messenger RNA
OC	oral cancer
OED	oral epithelial dysplasia
OLP	oral lichen planus
OR	odds ratio
ORF	open reading frame
OSCC	oral squamous cell carcinoma
PBS	phosphate buffered saline
PCR	polymerase chain reaction
pRb	protein retinoblastoma, tumour suppressor gene
p53	protein 53, tumour suppressor gene
RFLP	restriction fragment length polymorphism
RLB	reverse line blotting
rpm	revolutions per minute
s	second
TAE	tris acetate EDTA
TBE	tris borate EDTA
TP53	wild type of p53
UV	ultraviolet

List of Figures

List of Appendix

List of Tables

Chapter 1

Introduction

1.1 OVERVIEW OF HUMAN PAPILLOMAVIRUS

1.1.1 General features

Human papillomaviruses (HPVs) are small DNA tumour viruses of approximately 55 nm in diameter (Figure 1.1), and up to 200 different types and many more sequences that are less well characterized have been identified (Brenna and Syrjanen, 2003). These viruses are classified, according to their tissue tropism, into dermatotropic and mucosotropic groups (Van Ranst *et al.*, 1992). Moreover, human papillomaviruses have the ability to cause both genital and extra-genital infections. The skin is the most common site of extra-genital HPV infection but other extra-genital sites including the mouth, nose, oesophagus, larynx, trachea, and conjunctiva have been also involved (Karcioglu and Issa, 1997; Buchwald *et al.*, 1997; McKaig *et al.*, 1998). Lesions caused by some types of HPV are benign, while malignant lesions are associated with other HPV types, thus, HPV can be classified also by their oncogenic potentials as low-risk and high-risk types (zur Hausen, 1991).

Figure 1.1 Idealised electron-microscopic depiction of HPV
Adapted from: http://www.hhmi.org/news/popups/hpv_pop1.html

1.1.2 Virology

Human papillomavirus comprises a group of non-enveloped viruses of the papovaviridae family, with icosahedral capsids that replicate their genomes within the nuclei of infected host cells. The double stranded, circular DNA genomes of all HPVs are approximately 8 kb in size. The viral genomes carry an average of nine major open reading frames (ORFs) (Table 1.1) (Molijn *et al.*, 2005), and these are generated from polycistronic mRNAs transcribed from a single DNA strand. The early region makes up approximately 45% of the viral genome, the late region 40%, and a non-coding region containing viral regulatory elements make up the remaining 5% (Sanclemente and Gill, 2002; Hafkamp *et al.*, 2004).

The early genes serve to regulate the transcription of DNA, while the late genes encode for proteins involved in viral spread, such as capsids proteins. A non-coding region of about 1 kb in size, known as the upstream regulatory region (URR) or the long control region (LCR), separates the early and the late region gene clusters. The early genes include E1, E2, E4, E5, E6 and E7, while the late genes are L1 and L2. Since the sum of the sizes of all genes exceeds the coding capacity of the genome, a certain amount of overlap of genes is expected and has indeed been found (Figure 1.2). Two late genes encode structural proteins of the virions: the L1 protein is the major capsid protein and L2 protein is the minor capsid protein. The URR contains the origin of DNA replication, promoters and other transcription regulatory elements. E1 and E2 proteins are two important regulatory proteins for HPV; their functions are essential for viral DNA replication and

thus permissive HPV infection. E6 and E7 proteins are important for host DNA synthesis (Severson *et al.*, 2001).

It has been demonstrated that a virus with less than 90% homology in the L1 and E6 genes and URR to known types is defined as a new type, one with 90% - 98% homology is categorized as a subtype, and one with >98% homology defined as a variant (Severson *et al.*, 2001; Chen *et al.*, 2005a).

Table 1.1 Summary of the functions of human papillomavirus (HPV) open reading frames

Gene	Function
E1	DNA helicase activity, DNA-dependent ATP-binding, ATPase activity. Role in replication and replication repression
E2	Regulate of viral transcription and replication, control of early region viral gene expression, necessary for efficient viral DNA replication together with E1
E3	No known function (only present in a minority of papillomavirus)
E4	Expressed as a late gene primarily in differentiating epithelium, role in productive infection, associated with the keratin cytoskeleton of cultured epithelial cells, role in viral regress
E5	Transforming activity in HPV-16 *in vitro*. Presumably stimulates benign cell proliferation *in vivo* but might have a role in the initiation of carcinogenesis
E6	Role in transformation process together with E7. Transcriptional activation properties. E6 of high-risk HPVs inactivates p53 by inducing its degradation. Together with E7 provides a cellular environment for viral DNA replication
E7	Transactivating properties similar to the adenovirus E2 promoter, induces DNA synthesis in quiescent cells, role in rodent cell transformation in co-operation with an activated *ras* oncogene. E7 binds to the hypophosphorylated form of the retinoblastoma protein (pRB) resulting in its functional inactivation permitting cell progression to S phase of the cell cycle. E7 proteins from the low risk HPV types 6 and 11 bind less efficiently than the E7 protein from high risk HPVs (types 16 and 18)
L1	A major capsid protein
L2	A minor capsid protein

(Adapted from Sanclemente and Gill, 2002)

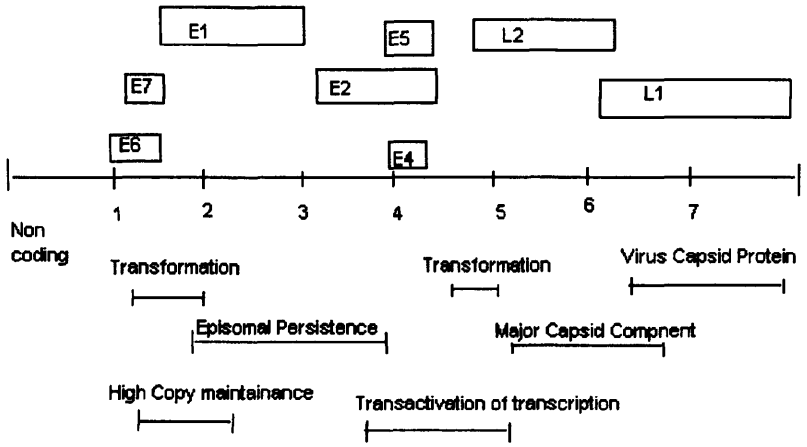


Figure 1.2 Genome organizations of papillomaviruses. Open bars represent open reading frame, which are labelled E or L depending on their position in the early or late region of the genome. (Adapted from Baron *et al.*, 1996)

1.1.3 HPV life cycle and biology

The papillomavirus life cycle requires the availability of epidermal or mucosal epithelial cells that are able to proliferate, i.e., basal layer cells. In these cells, viral gene expression is largely suppressed, although the limited expression of specific `early` viral genes (such as E5, E6, E7) results in enhanced proliferation of the infected cells, where the infected cells divide (zur Hausen, 2002) (Figure 1.3). Following entry into the suprabasal layers, `late` viral gene expression is initiated; the circular viral genome is then replicated and structural proteins form.

The viruses may have two modes of replication, either by stable replication in low copy number in episomal form in the proliferating basal cells or by replication taking place in terminally differentiated layers productively with the infected cell dividing repeatedly to form a wart (Garland, 2002). Upon

cell division, a daughter cell will remain as a part of the basal layer, while the other daughter cell will migrate outwards to start differentiation. At this stage, HPV-DNA segregates with the two daughter cells and replicates to sustain the maintenance of 50-100 copies of the genome per cell. In the upper layers of the epidermis or mucosa, complete viral particles are assembled and released (Severson *et al.*, 2001; zur Hausen, 2002).

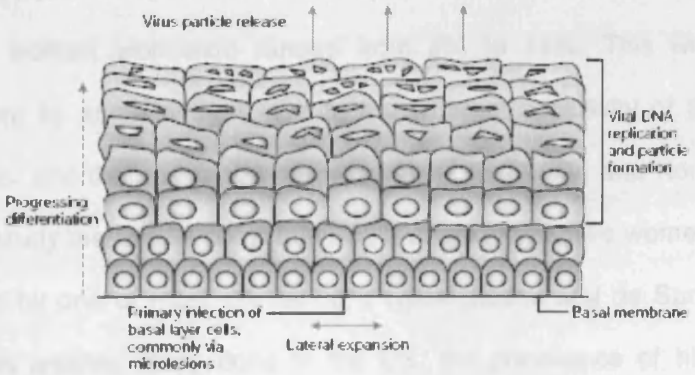


Figure 1.3 Human papillomavirus life cycle, Infection takes place in proliferating tissue, through a microlesion of the mucosa or the skin. Some of the infected cells migrate to the suprabasal layer, where viral genes are activated. Virions are released at the uppermost surface. (Adapted from zur Hausen, 2002)

1.1.4 Epidemiology of human papillomavirus infections

HPV is a very common infection; it can infect individuals without any clinical manifestation. Thus, only small fraction of infected patients will progress to invasive cancer. HPV has been classified to high risk, intermediate and low risk types (Table 1.2) (Baseman and Koutsky, 2005; Chen *et al.*, 2005a).

Table 1.2 Showing classifications of HPVs according to their risk

Risk classification	HPV types
High-risk	16,18,31,33,35,39,45,51,52 56,58,59,68,73,82
Probable high-risk	26,53,66
Low-risk	6,11,40,42,43,44, 54,61,70,72,81,CO6108
Undetermined risk	34,57,83

(Adapted from Baseman and Koutsky, 2005; Chen *et al.*, 2005)

It has been estimated that the population prevalence of HPV infection among women worldwide ranges from 2% to 44%. This wide variation explained by some authors due to the different sensitivity of the detection methods, and the age range of the samples (Baseman and Koutsky, 2005). In one study the results show that >50% of sexually active women have been infected by one or more genital HPV types (Bosch and de Sanjose, 2003). While in another study done in the US, the prevalence of high-risk HPV infection among women, with mean age of 25 years, was 27% (Kulasingam and Myers, 2003). A recent study in Scotland, women attending a routine cervical cancer screening (mean age: 36.6 years), showed that the prevalence of PCR-detected HPV-DNA was approximately 20.5% for all HPVs and 15.7% for high-risk HPVs (Cuschieri *et al.*, 2004). Although HPV type 16 is the most common type among cervical cancer cases, it is also the most common type found in cytologically normal women (Schiffman, 1992a; Woodman *et al.*, 2001; Munoz *et al.*, 2003).

Data from multiple international studies show that the median age of oncogenic HPV prevalence among all women is 15%, while the median oncogenic HPV prevalence among women age 30 and older is 9% (Bosch and de Sanjose, 2003). It appears that the prevalence of HPV infection is highest among young women, decreasing with increasing age (Schiffman, 1992b). Other studies have shown a peak prevalence of HPV infection in women below 25 years of age, with a decrease among women aged between 35-54 years and a second peak of prevalence was reported to be after the age of 55 years (Herrero *et al.*, 2000).

Other studies suggested that the incidence of HPV infection with oncogenic types is higher than that of the non-oncogenic HPV (Ho et al., 1998; Giuliano et al., 2002; Richardson et al., 2003). The cumulative incidence of high-risk HPV infection among women aged 15-19 years in England was found to be 44% over a 3-year period of time, which increased to 60% after 5 years (Woodman et al., 2001). Studies conducted in the US showed similar results among women within the same age group (Ho et al., 1998; Winer et al., 2003; Baseman and Koutsky, 2005).

1.2 Genotypes of Human papillomavirus

To date, up to 200 types have been identified (Garland, 2002; Munger *et al.*, 2004). HPV is classified according to genetic similarity with identified types, or grouped according to their tissue tropism (dermatotropic or mucosotropic), and as outlined earlier, by their potential to induce malignant transformation (Brentjens *et al.*, 2002). HPV types that are found preferentially in cervical and other anogenital cancers have been designated

as `high-risk` types. Conversely, those found primarily in genital warts and non-malignant lesions are considered as `low-risk` (Ostwald *et al.*, 2003) (Table 1.3).

Genotypically, papillomaviruses may also be divided into supergoups A-E, each with various subgroups (Chan *et al.*, 1995). The differences and similarities among HPV types are graphically represented as phylogenic trees as in Figure 1.4. Genital and mucosal HPVs belong to supergroup A, although typical genital viruses are also found in oral or laryngeal mucosa. Supergroup B contains most of the HPVs associated with epidermodysplasia verruciformis (EV), while supergroup E contains other human and animal HPV types. The differentiation of supergroups A and B was supported by their differences in natural history based on morphology, high bootstrap scores of phylogenetic trees and biological differences in gene regulation, e.g., similar *cis*-responsive elements being shared by types of the same supergroup (Tan *et al.*, 1994). Supergroups A6, A7, and A9 include viruses frequently demonstrated in genital high-grade dysplasia and carcinomas, leading to the concept of low and high-risk HPV infections (zur Hausen, 1987). Intragenomic variability has also been observed in different HPV types. Approximately 2% of sequence variability takes place in the coding region, while 5% occurs in the non-coding region (Campione-Piccardo *et al.*, 1991).

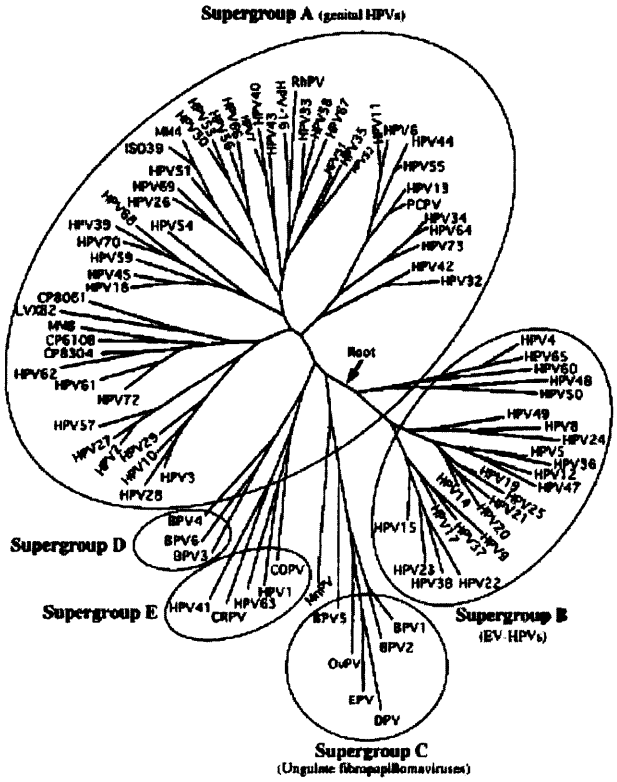


Figure 1.4 A phylogenetic tree of 92 HPV types based on weighted parsimony evaluation of the 291 bp L1 segment, showing supergroups A, B, C, D and E. (Adapted from Chan *et al.*, 1995)

Table 1.3 Summary of some of the different HPV genotypes and the disease associated

	Clinical manifestation	HPV type
Cutaneous lesions		
skin warts		
	Plantar warts	1,2,4
	Common warts	2,4,26,27,29,49,57
	Flat warts	3,10,28,41,49
	Butchers warts	7
	Epidermodysplasia verruciformis (EV)	3,,5,8,9,10,12,14,15,17,19-25,36,47,50
	Squamous cell carcinomas (actinic keratosis)	16
	Squamous cell carcinomas (keratoacanthoma)	37
	Squamous cell carcinomas (immunocompromised)	48,60
Mucosal lesions		
Anogenital warts		
	Condyloma acuminatum	6,11,16,18,30,42,43,44,55,70
Anogenital carcinoma		
	High risk	16,18
	Intermediate risk	31,33,35,39,45,51,52,56,58,59,68,73
	Low risk	6,11,26,40,42,43,44,53,54,55,62,66
Aerodigestive lesions		
	Recurrent respiratory papillomatosis	6,11
	Nasal papillomas, dysplasias, carcinomas	57
	Laryngeal condylomata	6,11
Oral lesions		
	Focal epithelial hyperplasia (Heck's disease)	13,32
	Vermillion warts	2
	Squamous papilloma	6,7,11,16,32
	Condyloma acuminatum	6,11
	Verruca vulgaris	6,11
	Oral hyperplasia	58
	Oral leukoplakia	16,18,33
	Oral lichen planus	6,11,16,18,31
	Proliferative verrucous leukoplakia	18
	Oral epithelial dysplasia	16
	Oral squamous cell carcinoma	16,18,33,45
Conjunctival papilloma		6,11,16
Tonsillar carcinoma		16

1.2.1 Serological evidences and serotyping

The concept of serotype has been useful in characterizing human viral pathogens, and in designing a protective vaccine against them. Difficulties in propagating HPV virions in cultured cells have obstructed efforts to identify and characterize viral immunologic variants (Kirnbauer *et al.*, 1992; Volpers *et al.*, 1994; Giroglou *et al.*, 2001).

There are many reasons for HPV serological assay to be laborious. First. there are over 200 different types, many of which are not malignancy associated and not sexually transmitted, so serological cross-reactions are hard to predict based on DNA homology. Second, most of HPV-infections are rapidly cleared spontaneously. Thus, many people testing negative for HPV-DNA may have had a previous infection. Third, in cutaneous HPVs, seroconversion can appear many months after infections. Also for genital HPVs, seroconversion can be delayed many months after the detection of viral DNA. Finally, testing for HPV genome of samples taken from the uterine cervix will not detect infections at other sites in the body (Dillner, 1999).

The relation between papillomavirus genotypes and serotypes remains an important issue for vaccine development, e.g., antibody–mediated neutralization of infectious virions appears to be genotype-restricted, i.e., certain types have demonstrated relatively unique capsid antigenic features, while others have demonstrated an ability to participate in cross-neutralization. It may be that certain genotypes denote unique serotypes, while others fall within serogroups consisting of two or more

related variants (Christensen *et al.*, 1994; White *et al.*, 1998; Giroglou *et al.*, 2001).

Of note, serum immunologic markers in HPV have not routinely been used in identifying the virus in acute or chronic diseases. The antibodies to HPV remain detectable for many years following infection, hence, serology is not suitable for distinguishing present and past infections (Molijn *et al.*, 2005). In addition, viral subtyping of HPV by serology is problematic as the capsid proteins of different HPV subtypes are antigenically quite similar (Garland, 2002).

Several studies which have investigated the presence of antibodies against L1, L2, E2, E4, E6 and E7 of high-risk group genotypes (mostly HPV 16 and 18) in cervical cancer patients, indicate that a humoral immune response to the virus does occur in such individuals (Zumbach *et al.*, 2000; Rosales *et al.*, 2001; Bosch *et al.*, 2002; Chang *et al.*, 2005). The frequency of seropositivity is higher in patients with HPV-associated genital lesions compared with appropriate control subjects. It has been postulated that the presence of the IgG antibody to the HPV-16 L1 capsid protein is an indication of persistent infection, and the presence of an antibody against HPV-16 E6 and E7 predicts HPV-associated malignancy (Chen *et al.*, 2005a). Also, it has been suggested that serum antibodies against HPV-16 L1, E6 and E7 are associated with increased risk for HNSCC and that they correlate with the presence of HPV-16 DNA in oral tumour specimens (Schwartz *et al.*, 1998; Zumbach *et al.*, 2000; Mork *et al.*, 2001).

1.3 Immune response to HPV

Human papillomaviruses are atypical viruses, as they cause no systemic infection. They are non-lytic (there is therefore no viraemia) and lesions with a productive viral infection do not have an associated inflammatory response or any significant histological evidence of immune activity. However, it is evident that the immune system is important in controlling HPV infections, both the specific immune response (humoral immune response) and the adaptive immune response (cellular immune response) being involved in the process (Sanclemente and Gill, 2002).

1.3.1 Humoral immune response

The human antibody response to HPV capsid antigen is type-specific, reacts mainly against conformational epitopes, and develops more than 8 months after productive infection occurs. Systemic anti HPV-IgG levels usually correlate with persistent HPV infection, while IgA against HPV-16 may exert some protective role against HPV infection (Carter *et al.*, 1996; Hagensee, 2000). It has been suggested that HPV-L1 antibody levels may fade away over time, but no changes in HPV-16 antibody levels have been detected in women followed up for 4 years (Hagensee, 2000). In addition, the increased rate of seroconversion among women with prevalent HPV-16 infection may be related to the fact that a higher percentage of prevalent versus incident HPV-16 infections are likely to be persistent (Carter *et al.*, 2000). However, once an infection has occurred, antibodies are not usually able to eliminate the virus, particularly if the virus is capable of entering a

latent state in which its DNA is integrated into host chromosomal DNA (Sanclemente and Gill, 2002).

1.3.2 Cellular immune response

It has been postulated that escape from immune-surveillance mechanisms is an important step in the progression of HPV tumourogenesis (zur Hausen, 2002). Activated Th1 responses seem to be important in controlling HPV via the production of cytokines, including interferon (IFN) and interleukin (IL-2). Interferon-α acts directly to eliminate virus by inducing an antiviral state in cells, while IL-2 acts indirectly by assisting the activation of cytotoxic T-cell lymphocytes (CTL). Both IFN-γ and IL-2 (activate natural killer cells (NK)) are important in the first few days of infection until a specific CTL response develops. In most viral infections, a CTL response develops within 3-4 days. As CTLs destroy infected cells, they also eliminate potential sources of new infection (Sanclemente and Gill, 2002).

1.4 Diagnostic aids and detection methods

Human papillomavirus is a particular difficult virus to study from an epidemiologic point of view. The low incidence of clinical manifestations, the inability to grow HPV in tissue culture and the exclusive species specificity of HPV, each limits the success rates in studying the virus among infected people with HPV.

There are many methods by which HPV can be detected, each having strengths and weaknesses.

1. 4.1 Direct Visual Inspection

The first step in diagnosis is the clinical assessment (sometimes termed as the visual test, or direct visual inspection (DVI)), where manifestations of HPV become visible. However, in only a minority of persons infected with the virus have early clinical signs of disease (Garland, 2002; Zanotti and Belinson, 2002).

In the cervix, early HPV lesions may appear white after staining with acetic acid (vinegar). Likewise, vital staining with Lugol's iodine may aid identification of HPV-associated diseases. The advantage of such methods is that they entail no need for laboratory processing of lesional material and the result is immediate. However, it does necessitate careful clinical evaluation by an experienced clinician (ACCP, 2004).

1. 4.2 Papanicolaou smear test

The Papanicolaou smear test is one of the most effective indicators of common and potentially serious clinical manifestations of HPV infection in women. It detects abnormal cells in a sample taken from the cervix. It involves performing a speculum examination to expose the cervix and collecting cervical cells using a wooden or plastic spatula or brush. These cells are then smeared and fixed on a glass microscope slide. These slides are transported to a laboratory where they are usually processed, and examined under the microscope. Histopathological examination of the smear then takes place. Cytological changes such as koilocytes, nuclear atypia, delayed maturation, hyperkeratosis, and parakeratosis are all highly associated with HPV infection and identify neoplastic cervical precursor

lesions with a high degree of accuracy. The success of the "Pap" smear in cervical cancer screening programmes has served as a model for population-based screening, early detection, and treatment (Garland, 2002; Zanotti and Belinson, 2002). The disadvantage of such procedures is the multistage process which can take several weeks before the results are available (ACCP, 2004).

Abnormalities found by the "Pap" smear are usually further evaluated by colposcopy, which involves high-powered illuminated magnification of the cervix using a colposcope (a binocular magnifying instrument). This enables the operator to determine the extent of any lesion, to taking biopsies and to provide treatment with cryotherapy or loop electrosurgical excision procedure (LEEP). Colposcopy is non-invasive and performed as an outpatient procedure. It does not require anaesthesia, but it is an expensive procedure, requiring appropriately trained clinician (ACCP, 2004).

Direct punch biopsies taken from colposcopic abnormalities are fixed in formalin and processed in H&E sections for light microscopy. On histological examination, the lesions are graded using the UN nomenclature and categorized as cervical intraepithelial neoplasms (CIN). The other morphological evidence for HPV infection (koilocytosis, dyskeratosis, multinucleation) also recorded (Branca *et al.*, 2004).

1. 4.3 Serum immunologic markers

As mentioned in Section 1.3, the detection of antibodies to HPV is not always diagnostically helpful. However, enzyme-linked immunosorbent assays (ELISA) tests for serum antibody against HPV proteins have been

developed which correlate well with the presence of HPV-DNA in the cervical samples (Kirnbauer *et al.*, 1994), and it has been suggested that the antibodies detected by ELISA are highly HPV-type-specific (Zumbach *et al.*, 2000). Of note, in the absence of HPV positive samples, it is impossible to pinpoint the anatomic site of the infection. Furthermore, antibody presence is not necessarily indicative of active infection, latent integration or oncoprotein production. It has been claimed that HPV seropositivity may be an indication of possible risk of oral cancer (Ha and Califano, 2004).

1.5 Molecular detection methods for HPV

A variety of molecular methods has been developed for the detection of HPV-DNA. All vary in their sensitivity, specificity and complexity. (Figure 1.5). It is worth to mention that HPVs can not be adequately cultured *in vitro* since it needs proliferating tissues, and serological assays only have limited accuracy since they can not distinguish between currently existing and passed-off infection.

1. 5.1 *In situ* hybridization (ISH)

In situ hybridization (ISH) permits the site of HPV-DNA within a cellular tissue to be identified. It involves the use of type-specific radioactively or enzymatically labelled DNA probes complementary to a particular HPV sequence. *In situ* and filter *in situ* hybridization (FISH) do not require DNA isolation from tissue but rather probe directly for the presence of viral sequence in tissues and smears (McKaig *et al.*, 1998). The sensitivity of this assay is of the order of 20-50 copies per cell (Syrjanen *et al.*, 1988).

However, it has been demonstrated that ISH depends upon the consistency of the complementary sequence present in the samples, and as the presence of the HPV-DNA in the oral cavity is known to be inconsistent. It has been demonstrated that the sensitivity of this assay to detect HPV in oral samples may not be high (Ha and Califano, 2004). Aside from the disadvantages of its low sensitivity and assay time, *in situ* hybridization affords the lowest specificity for the detection of HPV sequences in clinical samples (Hubbard, 2003).

1. 5.2 Dot blot hybridization (DB)

Both Dot blot hybridization and Southern blot hybridization methods require the isolation and purification of cellular DNA from a specimen. Dot blot hybridization is a rapid technique that can be used to screen multiple specimens and requires small quantities of DNA. It does not require digestion of the DNA sample with restriction enzymes or gel electrophoresis before hybridization. Instead, extracted DNA is denatured, bound to membrane and hybridized with specific HPV-DNA probes. It is sensitive to about one copy of cellular DNA per genome, but not as specific as Southern blotting and cannot distinguish between episomal or integrated HPV genomes (McKaig *et al.*, 1998).

1.5.3 Southern blot (SB)

Southern blot has long been one of the principal methods for the detection of HPV-DNA. It offers the ability to distinguish between episomal and integrated DNA, and it can detect up to 0.1 copies *per* cell (Syrjanen, 1990). Southern blot may have less sensitivity than PCR (Schiffman, 1992b;

Frazer *et al.*, 1993; McKaig *et al.*, 1998), and requires the use of radioactive probes, it is labour intensive and is thus expensive (Zanotti and Belinson, 2002).

1. 5.4 Hybrid capture-2 (HC2)

Hybrid capture-2 (HC2) is based upon hybridization in solution of long synthetic RNA probes complementary to the genomic sequence of 13 high-risk and 5 low-risk HPV types. This method has the advantages of high sensitivity, ease of performance in clinical sittings, and suitability for automation for genotyping. It does not require special facilities to avoid cross contamination, and does not require the use of radioactivity, in addition, it is commercially available. Cross reactivity of HC2 high-risk probes to HPV types that have a significant risk for cervical cancer may be considered as an advantage in identifying persons with high-risk HPV infection, but cross-reaction with low-risk types may cause false positive results that can lessen the specificity of the assay. Recently, HC2 has been approved by Food and Drug Administration (FDA) for *in vitro* diagnostic use (Konya *et al.*, 2000; Vernon *et al.*, 2000; Zanotti and Belinson, 2002; Iftner and Villa, 2003).

1.5.5 Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) is a valuable tool because it allows *in vitro* multiplication of unique regions of DNA so they can be detected within a large background. The sensitivity and specificity of PCR-based assays are varied, depending mainly upon the primer sets used, the size of the PCR product, reaction conditions, the performance of the DNA polymerase used in the reaction, the spectrum of HPV-DNA amplified and ability to detect

multiple types (Iftner and Villa, 2003). This technology can be used for viral detection, viral load quantitation, DNA sequencing, and mutation analysis and can also be performed in multiplex, whereby multiple target DNA sequences can be analyzed simultaneously (Hubbard, 2003). The commonly used PCR assays have a sensitivity of 1 to 10 virions *per* sample (Garland, 2002). Due to this high sensitivity, care must be taken to ensure the amplified target does not contaminate negative specimens and lead to false positives (Hubbard, 2003). In general, the efficiency of the PCR assay decreases with increasing amplicon size. Consequently, the efficiency of PCR primers generating a small product is considerably higher than primers yielding larger amplicons (Park *et al.*, 2004; Molijn *et al.*, 2005).

The use of consensus or general primer-mediated approach allows the detection of a broad spectrum of HPV genotypes in one assay. The first generation consensus PCR is designed to detect mucosotropic HPVs, and uses primers (MY 09/11 and GP^{+} 5/6) which anneal to highly conserved region L1 open reading frame (Remmerbach *et al.*, 2004). In this particular assay, the primers or the PCR conditions are adapted to accommodate detection of as many different HPV types as possible (Qu *et al.*, 1997; Garland, 2002; Castle *et al.*, 2002).

The use of consensus primers vs. specific primers would theoretically result in higher detection rates, as many different types of HPV can be identified. However, an advantage of using primers for E6 and E7 is that they allow the detection of the high-risk types of HPV (Harnish *et al.*, 1999; Chow *et al.*, 2000; Sasagawa *et al.*, 2000; Venturoli *et al.*, 2002). A modification of MY 9/11 to the PGMY 09/11 primer system has been developed which has

addressed some of the limitations of these traditional degenerate primer sets. The PGMY primer set has a significantly increased ability to detect multiple infections as it amplifies certain HPV types that are inefficiently detected by using the MY 09/11 set and thus detects approximately 10% more HPV positives samples (Gravitt *et al.*, 2000; Garland, 2002; Coutlee *et al.*, 2002). Another modification is the use of short PCR fragment primers (SPF), which enables the generation of a smaller amplicon, which has the advantage of more efficiently detecting HPV-DNA in formalin-fixed paraffin-embedded materials and archival Pap smear than other sets of previously mentioned primers (Garland, 2002; Perrons *et al.*, 2002).

Viral load quantitation using PCR technologies has become a methodological challenge since it has been suggested that high viral copy numbers correlate with increased risk of development of HPV-associated cervical lesions (Ha *et al.*, 2002; Kreimer *et al.*, 2005). Real-time PCR or Real Quantitation PCR (rt-PCR or RQ-PCR) represents perhaps the best approach to target nucleic acid quantitation. This technique allows continuous monitoring of PCR products, since dual-labelled fluorigenic probes emit fluorescence as the PCR reaction proceeds. Reactions are performed in well plates without the need to analyze the PCR products on gel, making it a useful tool for simultaneous testing of a large number of samples. This method has the advantage of being reproducible, rapid and applicable in clinical settings. Additionally, reactions can be run in multiplex with the use of different fluorochromes, such that, the starting concentrations of several target DNAs can be analyzed at once (Ha *et al.*, 2002; Hubbard, 2003). However, like any PCR based methodology, this technique is subject

to variations according to the primer sequence, target DNA and method of storage of the study tissues. Moreover, it requires expensive equipment and reagents. Nevertheless, its use in epidemiological studies seems to be warranted by the information provided. Unfortunately, the true clinical relevance of viral load measurement may be overshadowed by sampling error in the collection of tissue specimens, e.g., difficulty in controlling the lesion size and proportion of infected cells to normal cells (Strauss *et al.*, 2000; Gravitt *et al.*, 2003; Iftner and Villa, 2003; Pretet *et al.*, 2004).

Major limitations to this method include the fact that the amount of cancer cells in a biopsy specimen may affect viral quantitation, and a considerable variability may exist in viral load measurements within a single tumour. Additionally, the viral load results may be either due to a measurement of a few cells with greater number of HPV-DNA copies, or many cells with few copies (Klussmann *et al.*, 2001; Kreimer *et al.*, 2005). Recently, however, a variation of rt-PCR that uses self probing amplicons known as Scorpions has been developed for detection, typing and quantification of more than 40 different HPV types in clinical samples (Hart *et al.*, 2001; Gravitt *et al.*, 2003; Iftner and Villa, 2003).

1.6 Genotyping

Once PCR using consensus primers has been performed, detection of individual genotypes can be maintained by several methods including restriction fragment length polymorphism (RFLP), reverse line blot hybridization (RLB) and direct DNA sequencing. An alternative approach is to use type specific PCR that are used to identify individual HPV type based on

E6 or E7 polymorphism (Clavel *et al.*, 1998; Kado *et al.*, 2001; Hubbard, 2003).

Restriction fragment length polymorphism methods (RFLP) are used to identify HPV genotype-specific restriction patterns derived from post-PCR consensus primers. The restriction enzymes used for most analysis are typically BamHI, DdeI, HaeIII, HinfI, and PstI. Human papillomavirus RFLP data are sometimes difficult to interpret, especially when mixed infections are encountered, and since RFLP are not in practice, positively identified by specific hybridization (e.g., Southern blot), identification of false bands can lead to major uncertainty in assigning genotypes (Astori et al., 1999; Grce et al., 2000; Kay et al., 2002; Hubbard, 2003).

For individual HPV typing, a non-radioactive reverse line blot hybridization system has been developed. This allows a rapid typing of consensus PCR products in a single hybridization step and requires only a limited amount of PCR product (Coutlee *et al.*, 1999; Jordens *et al.*, 2000; Laconi *et al.*, 2001; Koskinen *et al.*, 2003; Molijn *et al.*, 2005) (more details in chapter 2, section 2.3.8).

The gold standard for recognition of all HPV types in a clinical sample is DNA sequencing, either by direct sequencing of the fragment or after cloning into plasmids, this method is however, expensive and thus has limitations for use as a diagnostic tool (Feoli-Fonseca *et al.*, 2001; Garland, 2002; Hubbard, 2003). Moreover, direct sequencing is not appropriate in instances of multiple HPV genotypes in clinical samples. Sequences that represent a minority genotype in the total PCR product may remain undetected and thus may underestimate the prevalence of infections with

multiple HPV genotypes. Comparing DNA direct sequencing with hybridization, the former detects multiple types in only 2% of samples, while reverse hybridization will identify multiple types in 25% of samples (Molijn *et al.*, 2005).

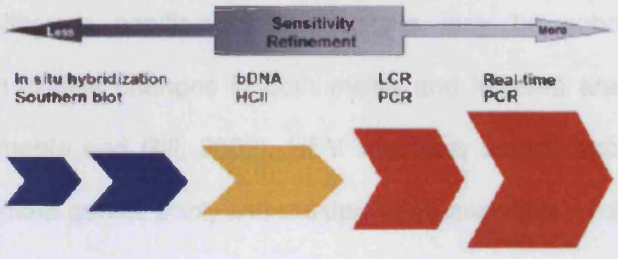


Figure 1.5 Relative sensitivities of different nucleic acid analysis methods. (Adapted from Hubbard, 2003)

1.7 Clinical associations

There are three types of HPV infections: latent, subclinical and clinical. In latent infection, HPV-DNA is present in host tissue and can be detected by molecular biological techniques, but causes no cytological abnormalities or clinical signs. In subclinical infection, there are histopathologically detectable cellular changes but no clinically detectable signs of disease. Finally, clinical HPV infection gives rise to both microscopically and macroscopically detectable disease (Severson *et al.*, 2001).

A variety of benign papillomavirus lesions of the skin and squamous mucosa are caused by human papillomavirus. These include common and plantar warts, flat warts, anal and genital condylomata acuminatum, cervical flat warts, macular pityriasis-like lesions in patients with epidermodysplasia verruciformis, oral papillomas and juvenile laryngeal papillomas. Laryngeal

papillomas are of concern as they occur in young children, tend to cause acute respiratory obstruction and often recur (Severson *et al.*, 2001; Syrjanen, 2003).

1.7.1 Genital lesions associated with HPV

Although papillomavirus infections may be subclinical, the most common clinical changes in both males and females are anogenital warts (Sanclemente and Gill, 2002). HPV infections usually occur throughout the lower female genital tract, and multiple sites are often involved, including the cervix, vagina and in the vulvar region. Lesions termed cervical intraepithelial neoplasia (CIN) are strongly associated with HPV infection, reflect mild to moderate cervical dysplasia, and are typified by large round cells termed `koilocytes`. In men, anal condylomas and penile warts occurring separately or together have HPV aetiology. The male counterparts of CIN are penile intraepithelial neoplasia (PIN). The lesions generally occur at a much lower rate than CIN, but are highly associated with high risk HPV infection and can undergo malignant progression to penile carcinoma (Munger, 2002). It has been postulated that HPV plays a major role in the pathogenesis of anal and peri-anal cancer (Beckmann *et al.*, 1989; Palefsky *et al.*, 1991; Palefsky *et al.*, 1998).

1.7.2 Cervical cancer

1.7.2.1 Epidemiology of cervical cancer

Worldwide, approximately 500,000 cases of invasive cervical cancer are diagnosed each year. It is considered the second most common cancer in women worldwide following breast cancer, and it is also considered as the principal cancer of women in most developing countries, where 80% of all cases are recorded (Franco *et al.*, 2001; Garland, 2002; Munoz *et al.*, 2003). However, it is evident that this may be a preventable malignancy as the mortality associated with cervical cancer has decreased by over 70% in the last 50 years in the United States, principally due to the wide spread use of cytology-based screening (Saslow *et al.*, 2002; Jemal *et al.*, 2004) (Table 1.4).

1.7.2.2 HPV in cervical cancer

Genital HPV infections are generally acquired via sexual intercourse (Koutsky *et al.*, 2002). However, HPV can sometimes be detected in the cervix of virgins, suggesting other routes of transmission (Xi *et al.*, 2002). If women are carefully followed up, about 50% will be infected with genital HPV within 2 years of sexual debut (Kjaer *et al.*, 2001; Koutsky *et al.*, 2002). It is estimated that the lifetime risk of contracting a genital HPV infection is 80%; however, this leads to genital warts in only 5% of infected individuals. While abnormal cervical scrapes (i.e. contain abnormal cellular atypia) occurs in 35% of infected females, cervical intraepithelial neoplasia (CIN) develops in 25%, and invasive carcinoma in less than 1% of HPV infected women (Schiffman, 1992b; Kjaer *et al.*, 2001; Koutsky *et al.*, 2002). It has been demonstrated that most HPV infections and low-grade CIN lesion

spontaneously regress within 8 months following identification (Hinchliffe *et al.*, 1995; Wheeler *et al.*, 1996; Moscicki *et al.*, 1998; Ho *et al.*, 1998; Koutsky *et al.*, 2002). The odds ratios of HPV in cervical cancer has been suggested to range from 72 to 347 according to the HPV oncogen presence. (Munoz, 2000; Garland, 2002)

Epidemiological studies have also established that HPV infection is a central cause of intracervical carcinoma (ICC) and its precursor lesions (Walboomers *et al.*, 1999; Franco *et al.*, 2001; Bosch and de Sanjose, 2003). In particular, HPV types 16, 18 and 45 have been found to be the principal viruses associated with cervical malignancy. Recent studies have confirmed earlier reports that an increased HPV-16 viral load detected in a cervical smear is associated with the presence or development of CIN/3 cervical cancer (Bekkers *et al.*, 2004; Rousseau *et al.*, 2004).

HPV is thought to enter the cervical mucosa via microabrasions to the epithelium. In benign lesions (warts) and low-grade dysplasias, the virus exists in an episomal form in basal cells and more differentiated cells. It replicates only in the nuclei of infected cells and is not transmitted from one cell to another; rather, release of virus from infected cells is the result of degeneration of desquamating cells. At the cytological and histological level, the cytopathic effect of HPV is the development of `koilocytes`, a mature squamous cell with a large perinuclear halo and enlarged hyperchromatic nucleus. Precursor lesions reflect the spectrum of epithelial atypia induced by HPV infection, ranging from permissive viral growth and benign epithelial proliferation (low-grade dysplasia) to disordered cell growth, genetic instability and deregulated viral gene expression (high-grade dysplasia). The

target for infection by oncogenic HPV types is usually the basal cells of squamocolumnar junction of the transformation zone of the cervix (the part of the ectocervix where the epithelium undergoes transformation from columnar to squamous during a woman's lifetime), and this is where the neoplasia usually originates (Garland, 2002).

Table 1.4 Cervical Cancer Incidence World Wide

Region	No of cases, 1985 (Parkin et al., 1985)	No of cases, 1990 (Parkin et al., 1999)	No of cases, 2000 (Ferlay et al., 2001)	ncidence (Ferlay et al., 2001)		Age-specific incidence, year			
				CR	ASRW	15-44	45-54	55-64	65+
World	437 300	371 200	470 606	15.7	16.1	9.5	44.9	51.9	41.9
More developed	93 700	83 300	91 451	15.0	11.3	11.9	22.4	23.8	26.3
Less developed	343 600	287 900	379 153	15.8	18.7	9.0	53.6	65.0	53.8
Africa	51 500	52 500	67 078	17.1	27.3	11.0	71.5	100.5	95.4
Eastern	21 800	21 500	30 206	24.4	44.3	16.1	114.8	174.4	153.9
Middle	6600	5700	6947	14.4	25.1	8.5	54.0	73.3	137.4
Northern	6200	5200	10479	12.2	16.8	6.2	49.0	68.5	45.9
Southern	6600	6500	5541	23.2	30.3	15.5	67.8	98.5	118.2
Western	10 300	13 600	13 903	12.5	20.3	9.5	57.4	70.6	60.3
America	68 000	74 800	92 136	22.0	21	15.1	55.2	57.8	55.0
Caribbean	3000	5000	6670	34.8	35.8	17.7	82.7	102.1	155.6
Central	13 700	17 700	21596	31.7	40.3	22.5	111.7	109.9	136.1
South	35 300	36 900	49 025	28.1	30.9	16.8	85.5	90.2	101.4
United States and Canada	16 000	15 200	14 845	9.5	7.9	9.0	15.4	16.8	14.2
Europe	67 000	58 200	64928	17.2	13	14.1	26.3	26.5	28.1
Eastern	40 100	27 500	35482	21.9	16.8	17.8	34.5	34.9	36.6
Northern	6300	7600	6049	12.6	9.8	12.0	17.6	16.7	20.2
Southern	8600	9900	10 116	13.7	10.2	10.5	20.8	23.7	20.9
Western	11 900	13 200	13 282	14.2	10.4	11.3	20.5	19.6	25.1
Asia	249 000	183 400	245 670	13.6	14.9	7.2	44.0	52.8	39.6
Asia excluding China	170 800	158 700	212297	18.0	21.1	10.3	63.0	75.0	53.5
Eastern	94 200	42 500	51 266	7.1	6.4	2.6	18.4	18.9	25.4
China	78 200	24 700	33 373	5.4	5.2	1.7	17.2	16.1	19.1
Japan	9400	8500	11681	18.1	11.1	8.8	21.2	25.6	42.2
Other	6600	9300	6212	16.2	15.3	10.3	33.6	41.9	55.0
Southeasten	42 500	30 900	39 648	15.3	18.3	9.1	59.0	58.2	45.9
Southcentral	109 500	107 000	151 297	20.9	26.5	11.9	79.2	100.8	65.6
Western	2800	3000	3458	3.8	4.8	2.6	13.1	15.3	14.1
Oceania	1800	2100	2156	14.2	12.6	12.3	27.4	28.2	29.0
Pacific islands†	500	800	1078	29.1	40.3	23.1	91.2	107.1	167.8
Australia / N.Zealand	1300	1300	1077	9.4	7.7	8.6	15.5	14.8	16.6

CR= crude rates per 100 000; ASRW= age-standardized rates per 100 000. World standard population. All rates correspond to the most recent compilation of cancer incidence.
† Melanesia, Micronesia and Polynesia
Adapted from Bosch and deSanjose., 2003

1.8 Routes of transmission of HPV

Human papillomavirus is mainly transmitted by direct contact with the source. Thus, the incidence of the skin HPV infection is known to increase when schoolchildren first make body contact with one another. Epidemiological studies of cervical cancer have clearly documented that HPVs are transmitted by sexual contact (IARC, 1995; Bosch *et al.*, 2002; Waller *et al.*, 2005). Recent studies have shown that 50%-80% of male partners of women with either CIN or genital warts or who were attending a gynaecology referral clinic have lesions with clinical or morphological features consistent with HPV infection. The transmission of anogenital HPV-16 infections have been consistently associated with sexual contact and the prevalence of cervical HPV infections increases between the ages of 15 and 40 years; especially among persons with multiple sexual contacts (Severson *et al.*, 2001).

The relationship between the sexual behaviour of patients, regarding age of sexual debut, number of partners, the practicing of oral sex and any history of genital warts has been extensively studied. It has been reported that, high risk HPVs were detected more often in patients with more sexual partners than the controls. Also, a higher risk of infection is found among those reporting oral-anal contact (Smith *et al.*, 2000; Gillison and Lowy, 2004; Scully, 2005). Furthermore, one study has shown that husbands of uterine cervical cancer patients have a higher risk of developing both tonsillar cancer and cancer of the tongue (Hemminki *et al.*, 2000; Hafkamp *et al.*, 2004). Of note, it has been demonstrated that people with a history of oral sex show a four-fold higher chance of HPV infection than those without

(Ritchie *et al.*, 2003; Hafkamp *et al.*, 2004). Although, in a recent article it has been suggested tha oral sex had no association to oral HPV infection, indeed, they have proposed that a persistent oral HPV infection of the spouse may increase the risk 10-folds of persistent oral HPV infection in the other spouse (Rintala *et al.*, 2006). It has also been suggested that the oral cavity may play a role as a reservoir for HPV infection. The vertical transmission of mucosotropic HPV from mother to baby during natural childbirth has also been observed (Daling *et al.*, 1986; McKaig *et al.*, 1998; Severson *et al.*, 2001).

1.9 Mechanism of HPV pathogenesis

As discussed in Section 1.1.3, HPV must infect mitotically active cells to enable it to proliferate. The receptors for entry of the virus into these cells are currently unknown. However, heparan sulphate has been proposed as mediator of initial attachment of virions to cells (Joyce *et al.*, 1999; Giroglou *et al.*, 2001; Longworth and Laimins, 2004). Three possible paths of entry have been suggested; from the site of mucosal injury; via metaplastic epithelium and from the squamo-columnar cell junction (Szentirmay *et al.*, 2005). Cells in the basal layer consist of stem cells and transient-amplifying cells that are continuously dividing, which provide a reservoir of cells for the suprabasal region. Infection of these cells by HPV leads to the activation of a cascade of viral gene expression that results in the production of approximately 20 to 100 extrachromosomal copies of viral DNA per cell. This average copy number is stably maintained in undifferentiated basal cells throughout the course of the infection (Longworth and Laimins, 2004).

1.9.1 Integration of HPV-DNA

The progression from pre-invasive to invasive cancer may be related to the physical state of HPV-DNA (episomal, integrated or mixed). Many researchers have demonstrated that the integration of HPV-DNA into the host cell genome occurs early in cancer development, and it is an important step in malignant transformation. However, integration appears to occur more frequently in HPV-18 associated cervical cancer than in HPV-16 associated cervical cancer (Munger, 2002; Sanclemente and Gill, 2002; Ha *et al.*, 2002; zur Hausen, 2002). Integration usually disrupts or deletes either E1 or the E2 open reading frames, which result in loss of expression of the corresponding gene product and also to upregulation of the viral oncoproteins E6 and E7 (Motoyama *et al.*, 2004; Szentirmay *et al.*, 2005).

HPV genes that cause cancer are of two distinct types: oncogenes (e.g. E6 and E7), and tumour-suppressor genes (e.g. p53 and pRb). The two types have opposite effects in carcinogenesis. Oncogenes facilitate malignant transformation, whereas tumour-suppressor genes block tumour development by regulating genes involved in cell growth. The E6 and E7 proteins of high risk HPVs are essential for initiation and maintenance of the transformed state (Munger, 2002; Longworth and Laimins, 2004).

1.9.2 The E6 protein

The E6 proteins are of both high and low-risk types, and are approximately 150 amino acids in size. They can interact with the cellular wild-type p53 tumour suppressor protein, inducing p53 degradation (a well-characterized tumour suppressor that regulates the expression of proteins

involved in cell cycle control, including the cyclin kinase inhibitor). Normally, p53 prevents cells with damaged DNA from progressing through the cell cycle by arresting them at the transition from G_1 to the S phase, thus allowing time for DNA repair. In case of excessive damage to the DNA, the cell will undergo p53 and BAK-mediated apoptosis. Therefore, inactivation of p53 by E6 leads to deregulation of the cell cycle and promotes accumulation of mutations as well as chromosomal instability. E6 oncoproteins activates host cell telomerase (a ribonucleotide complex that synthesizes telomere repeat sequences), which is critical for cell immortalization during cell growth (Hafkamp *et al.*, 2004).

1. 9.3 The E7 protein

It has been demonstrated that the major function of E7 protein is the interaction with the cellular pRb protein (important as a regulator of the cell division). The binding of E7 protein to Rb results in pRb degradation (normally prevents cells from entering the cell cycle by inactivating E2F). It is known that E7 from the oncogenic HPV type 16 and 18 binds with pRb more strongly than does E7 from the benign HPV types. E7 appears to block the function of cyclin-dependant kinase inhibitor $p21^{WAF/CIP1}$ and to induce centriole amplification. These actions of E7 protein also contribute to tumourogenesis by stimulating cell cycle progression and the development of genetic instability and aneuploidy (Hagensee, 2000; Hafkamp *et al.*, 2004).

Loss of Rb function can also lead to activation of p53, which normally suppresses cell growth and induces or potentiates apoptosis. In many cancers, a combination of mutation and gene deletion leads to loss of p53

activity. However, in cervical cancer, the p53 gene is intact and the p53 protein is expressed in the transformed cells, but its activity is lost by binding to the HPV-E6 oncoprotein. The loss of p53 mediated apoptosis is thought to be an important part in tumour progression (Garland, 2002) (Figure 1.6).

It has been suggested that the differences between the ability of the low and high-risk HPV types to induce immortalization and transformation may depend on their abilities to interact with the cell cycle components, resulting in the loss of multiple cell cycle checkpoints that are essential in maintaining host genome fidelity, thus leading to potential accumulation of genetic abnormalities (Brenna and Syrjanen, 2003).

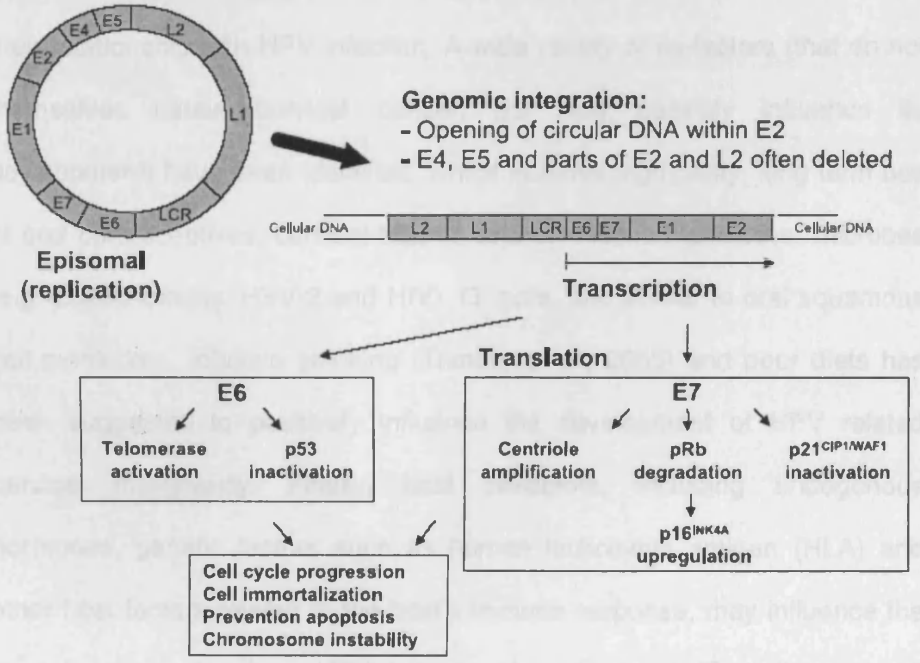


Figure 1.6 In premalignant lesions, the HPV genome is present as circular DNA in the nucleus of infected cells, harbouring the ORFs. During cancer development, the viral DNA becomes integrated into the host, cell genomic DNA, thereby frequently losing (parts of E2, E4, E5 and L2 genes). In most cases, the integrated viral DNA contains the intact E6 and E7 genes of which the expression is upregulated. (Adapted From Hafkamp *et al.*, 2004)

1.9.4 Co-factors in HPV carcinogenesis

As discussed previously, epidemiological studies have indicated that only a small percentage of infected women with oncogenic HPV types will progress to high-grade squamous intraepithelial lesions (HSIL) or cervical malignancy. Thus, it has been suggested that other factors acting in conjunction with HPV influence the transition from cervical HPV infection to malignancy (Castellsague and Munoz, 2003). A high percentage of infected women usually clear the infection by the immunological mechanisms (zur Hausen, 2002).

Many sexual behaviour parameters have been examined to determine their relationship with HPV infection. A wide variety of co-factors (that do not themselves cause cervical cancer, but may possibly influence its development) have been identified, which includes high parity, long term use of oral contraceptives, cervical trauma and co-infection with other microbes (e.g. *C.trachomatis*, HSV-2 and HIV). Of note, and similar to oral squamous cell carcinoma, tobacco smoking (Trimble *et al.*, 2005) and poor diets has been suggested to positively influence the development of HPV related cervical malignancy. Finally, host co-factors, including endogenous hormones, genetic factors such as human leukocytes antigen (HLA) and other host factors related to the host's immune response, may influence the risk of progression from HPV infection to malignancy (Castellsague and Munoz, 2003) (Figure 1.7).

Defective immunosurveillance certainly plays a role in the likelihood of females developing cervical malignancy. Women with HIV infection or AIDS

are known to be at an increased risk for HPV infection and cervical malignancy (Branca *et al.*, 2000; Branca *et al.*, 2004). HPV infection and CIN in HIV infected women can rapidly progress to high-grade CIN and cancer (Amit *et al.*, 2001; Hawes *et al.*, 2003).

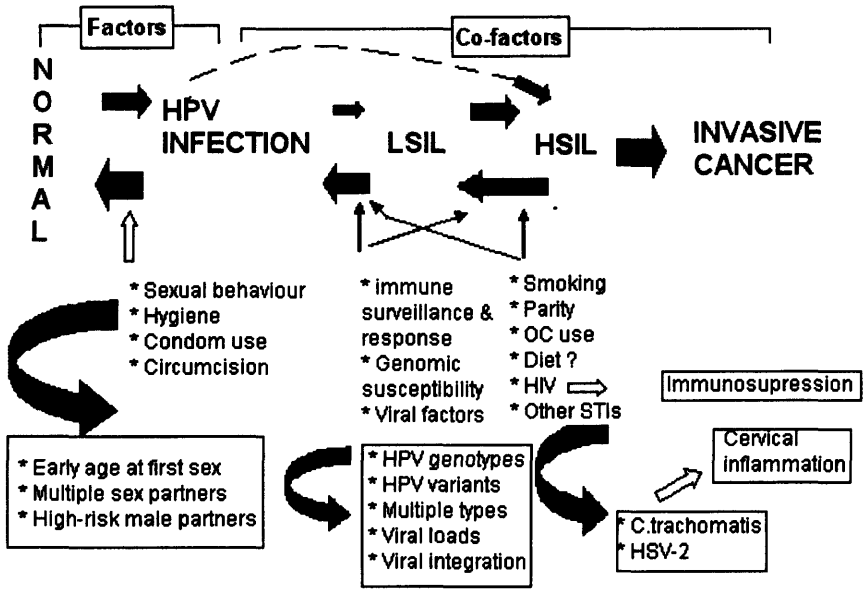


Figure 1.7 Factors and co-factors in cervical malignancy. (Adapted from Castellsague *et al.*, 2002)

1.10 Therapies

Generally, therapy of HPV infections can be divided into antitumour (i.e., anti-wart) and antiviral interventions. For the dysplastic and neoplastic lesions, surgery is the standard of care with adjunctive chemotherapy and/or radiotherapy for some malignancies. Treatment of benign lesions, especially non-anogenital warts, usually remains antitumour in most cases, chemotherapeutic, cytodestructive, surgery, etc. Such non-antiviral

interventions are also used in the majority of cases of condyloma acuminatum. Therapies that are neither antiviral nor immunomodulator remain the standard of care for most cases of non-anogenital warts and for a number of genital warts. Surgical excision offers the advantage of rapid removal of the warts but is associated with high recurrence rates as well as the potential for bleeding, scarring, and bacterial infection (Stone *et al.*, 1990). Electrosurgery may reduce the amount of bleeding but not the problem of recurrences. Treating warts with CO_2 laser can achieve excellent results, but recurrences rates can be as high as with other surgical treatments. Disadvantage of CO_2 laser is its high cost and the possibility of the presence of HPV-DNA in the laser plume (Ferenczy *et al.*, 1995).

Cryotherapy is a destructive treatment for warts that is bloodless but can be painful, and often requires a series of treatments over several weeks (Abdullah *et al.*, 1993). A number of chemotherapeutic/cytodestructive agents are available with different qualities. The logical approach to a viral infection such as HPV is to use an antiviral drug. This criterion is met by the interferon (IFN) α, β, γ as well as by Imiquimod and Cidofovir. Immunotherapies are promising because they are thought to enhance the effectiveness of local therapy while decreasing the recurrence rates (Severson *et al.*, 2001).

Interferon is a protein that interferes with viral replication; they have been identified as the body's first line of defence against most major viral diseases. Interferons are divided into Type 1 (α and β) Type 2 (γ). Type 1 shares the same membrane receptors, and is predominantly antiproliferative, while Type 2 has a distinct receptor, and act mostly as immunomodulators promoting cell-mediated immunity. Interferon α (IFN α) is a multigene product

formed by B-lymphocytes, null lymphocytes and macrophages in response to viral particles or nucleic acids. Interferon β (IFN β) is a single gene product formed by fibroblasts, epithelial cells and macrophages in response to viral particles to nucleic acids. In addition, Interferon γ (IFN γ) is a single gene product formed by T-lymphocytes in response to foreign antigens (Rockley and Tyring, 1995; Severson *et al.*, 2001).

Interferon alpha (IFN α) was the first antiviral agent approved by the United States Food and Drug Administration (FDA) for the treatment of condyloma acuminatum. Despite its efficacy and safety, IFN α never became widely used for genital warts because of its route of administration, side effects and cost. Imiquimod is an immune response modifier with antiviral activity that has been recently FDA approved for genital wart therapy. It is topically self-applied, safe, effective and appears to be associated to determine objectively the cost effectiveness of various therapies for condyloma acuminatum in the absence of head-to-head trials. Many studies showed 5% solution of Imiquimod was more effective than control (clearance rates 52% compared with placebo). Further studies showed that Imiquimod has induced tumour wart regression which was associated with local interferon and tumour necrosis factor α production (Tyring *et al.*, 1998; Hagensee, 2000).

1.11 Vaccines

There are three approaches to HPV vaccine development. The first approach is to block HPV-induced neoplasia by preventing the virus from establishing infection in the epithelium, mainly through the induction of

neutralizing antibody via viral capsid protein. A prophylactic vaccine should stimulate the complete neutralization of free virus upon exposure before infection can occur. However, this may be of less benefit to those individuals who already have established infection and dysplasia. The second approach of vaccine development addresses the needs of these patients by attempting to induce a cellular immune response, which may be able to prevent and induce the regression of neoplastic lesions. This strategy is known as antigen-specific immunotherapy. Finally, the third approach seeks to combine prophylaxis and therapy in one vaccine, in an attempt to provide total coverage for people who are newly exposed to high risk virus and for people with current infections and dysplasia (Devaraj *et al.*, 2003).

It has been demonstrated that a prophylactic or therapeutic vaccine may prevent HPV-associated HNSCC in the future. Since, a prophylactic vaccine composed of HPV-16 viral capsid proteins has shown promise for the prevention of persistent cervical infections (Koutsky *et al.*, 2002; Gillison, 2004).

In-vitro, production of HPV empty capsids has led to the development of vaccines against various types of HPV. Prophylactic vaccines against HPV will probably target the empty capsid as an antigen but will need to be multivalent to be effective. Although, therapeutic vaccines target is less well-defined, modified empty capsids may also be a vaccine candidate. HPV-16 E6 and E7 conjugated with L2 protein and assembled with L1 into empty capsids have the potential advantage of combining a prophylactic and a therapeutic vaccine (Hagensee, 2000).

1.12 Epidemiology of head and neck squamous cell carcinoma (HNSCC)

Head and neck squamous cell carcinoma (HNSCC) comprises a related group of cancers including neoplasms originating from the oral cavity, pharynx, larynx and oesophagus. Worldwide, HNSCC represent 6.5% of the annual cancer cases with an estimated number of new cases per annum of 38.3/100,000 in the US (Forastiere *et al.*, 2001). In recent decades, the incidence of HNSCC has increased in Western Europe and the US (Hafkamp *et al.*, 2004). Tobacco smoking, extensive alcohol drinking and betel quid chewing are well known risk factors in the aetiology of HNSCC. These factors are independent and it has been suggested that their effects are synergistic when combined (Lewin *et al.*, 1998; Forastiere *et al.*, 2001).

Some studies observed the effect of alcohol upon the development of HNSCC among smokers and non-smokers (Talamini *et al.*, 1990). Other studies have demonstrated the relation of HNSCC with the amount of both tobacco and alcohol consumption. An increased risk of HNSCC has been observed with both quantity of tobacco consumption, and the duration of habit. In addition, a decreased risk has been observed following long-term cessation of tobacco smoking. Similarly, a dose-relationship has been observed with increased alcohol consumption (Brennan and Boffetta, 2004).

1.12.1 The role of genetic factors in HNSCC

It has been demonstrated that in cervical oncogenesis, aneuploidization precedes integration of high-risk HPV genomes. Deregulated viral oncogene expression appears to result first in chromosomal instability and aneuploidization, this being followed by

integration of high-risk HPV genomes into cellular chromosomes (Cooper *et al.*, 2003; Melsheimer *et al.*, 2004).

Braakhuis and colleagues (2004) have recently demonstrated that HNSCCs with HPV type 16 DNA expressing E6 and E7 had significantly lower rates of loss of heterozygosity (LOH) at chromosomal regions 3p, 9p and 17p than tumours that contained inactive HPV-DNA (i.e. no expression of E6 and E7), they also observed a negative association between active HPV-DNA and mutations in TP53 (the gene encoding the p53 tumour suppressor). All of these genetic regions are known to contain tumour suppressor genes that are commonly deleted in HNSCC. It is assumed that LOH at these regions plays a role in HNSCC tumourogenesis. Hence, HNSCC associated with HPV would seem to involve oncogenic mechanisms different to that of non-HPV related tumours (typically caused by tobacco and/or alcohol).

Head and neck squamous cell carcinoma is occasionally featured in several inherited cancer syndromes, in particular, Fanconi's anaemia, a rare autosomal recessive disorder of DNA repair, characterized by congenital bony formations, progressive bone marrow failure and predisposition to leukemia and carcinomas (e.g. aplastic aneamia). Recently, it has been observed that patients with Fanconi's anaemia have a 500 to 700 fold higher incidence of HNSCC than the general population (Syrjanen, 2005). It is considered to be an excellent model for studying HPV and its role in carcinogenesis (Lowy and Gillison, 2003; Szentirmay *et al.*, 2005), particularly as HPV is often detectable in OSCC of patients with Fanconi's anaemia. The virus may be of aetiological importance for the development of

OSCC in these patients. However, a p53 polymorphism may also be of aetiological relevance (Kutler *et al.*, 2003; Gillison and Lowy, 2004).

1.13 Epidemiology of oral squamous cell carcinoma (OSCC)

According to world statistics, approximately 267,000 new oral cancer cases were diagnosed and about 128,000 patients died from this cancer in the year 2000, the high risk areas and countries include the Bas-Rhino region in France, the Vasi region in Hungary, the Hong Kong in China, India, Brazil and the Philippines (Ferlay J *et al.*, 2001). Cancer of the oral cavity is the sixth most prevalent cancer worldwide and is the third most common cancer in the developing countries and the eighth in developed countries. Of note, 90% of these oral cancers are squamous cell carcinoma (SCCs) (Syrjanen, 2005). The overall incidence and mortality associated with OSCC continues to rise. Even though a higher number of new cases of cancer occur in developed countries (1,3329000 vs. 787,000) a higher incidence of OSCC occur in developing countries (8.4 / 100,000 in men and 2.3 / 100,000 in women) than developed countries (5.8 / 100,000 in men and 3.3 / 100,000 in women) (Greenlee *et al.*, 2000; Carvalho *et al.*, 2004). Trends in 5-year survival have not changed since 1974, the overall survival being 53 % for the period between 1989 and 1995. Moreover, a recent analysis show that while survival is improving for all cancers, the greatest decline in mortality is associated with OSCC (Carvalho *et al.*, 2004).

1.14 Human papillomavirus in head and neck squamous cell carcinoma

Epidemiologic and molecular pathology studies provide substantial evidence that HNSCC is usually linked with tobacco and/or alcohol, or betel use, but there are group of individuals who develop HNSCC in the absence of exposure to these risk factors. The possibility of the involvement of microbial agents has been proposed. Syrjanen and colleagues, first described the involvement of HPV in the pathogenesis of HNSCC in 1983, when morphologic features consistent with HPV infection were identified within OSCC tumour cells. A study by the IARC (Herrero *et al.*, 2003) have demonstrated that HPV-positive HNSCC are more common in patients who do not smoke or chew tobacco, but persistent HPV infection and tobacco consumption combined are associated with an increased risk of HNSCC. In a recent systematic review, the overall prevalence of HPV in HNSCC was 25.9% only (Kreimer *et al.*, 2005), while the OR of HPV in HNSCC has been reported to range between (3.7-5.4) (Syrjanen, 2005).

It has been observed that HPV positive HNSCC show a statistical association among individuals with a history of multiple sexual partners, practicing oral sex and with a previous history of genital warts which suggest sexual transmission (Schwartz *et al.*, 1998; Herrero *et al.*, 2003). Due to the similarities of the mucosal lining in the head and neck region and that of the cervix, it is expected that only a small percentage of HNSCC patients with HPV infection will develop virus-associated malignancies as in cervical cancer, which indicates that other co-factors are involved in the progression from an infected cell to a transformed cell with invasive potential (Hafkamp *et al.*, 2004).

Human papillomavirus type 16 has been identified in approximately 90% of HPV positive HNSCC and is usually present in episomal, integrated and mixed forms (Herrero *et al.*, 2003; Gillison and Lowy, 2004; Chen *et al.*, 2005a). The presence of high-risk type in healthy oral mucosa suggests a dormant infection that might contribute to the development of oral cancer later (Sugerman and Shillitoe, 1997) or that not all HPV-16 lead to OC . A novel HPV-16 related virus has been proposed by Maitland *et al* (1989), they have claimed that this subtype is associated with HNSCC causing changes in the promoter-enhancer region that make them specifically active in oral keratinocytes (Scully, 2002).

The increased distinction between HPV-HNSCC and HPV-negative HNSCC supports the hypothesis of the two pathways in the pathogenesis of HNSCC (Figure 1.8), one driven by alcohol and tobacco, and the other by HPV. The molecular abnormalities found in HPV-HNSCC reflect the oncogenic function of viral E6 and E7. The molecular signature of HPV-HNSCC includes HPV-DNA, HPV-E6 and E7 expression, TP53, lack of promoter methylation of 14-3-3α, lack of RASSF1A, decreased cyclin D kinase (CDK), decreased pRb and upregulation of p16. By contrast, HPV-negative HNSCC is characterised by tobacco-induced p53 mutation, decreased expression of 14-3-3α and RASSF1A, increased CDK, normal or increased levels of pRb staining and decreased p16 due to deletion or mutation (Gillison, 2004) (Table 1.5).

Table 1.5 Clinical and molecular differences in HNSCC +positive HPV DNA and HNSCC – negative HPV DNA. (Adapted from Hafkamp *et al.*, 2004)

	HPV-positive	HPV-negative
Clinical characteristics		
Location	Oropharynx	All sites
Differentiation grade	Poor	Well
Basaloid appearance	Yes	No
Size at diagnosis	Smaller	Larger
Disease stage	More advanced	Less advanced
Patient's age	Younger	Older
Patient's tobacco intake	Less	More
Patient's alcohol intake	Less	More
Overall/disease-free survival	Longer	Shorter
Molecular characteristics		
HPV DNA	Yes	No
E6/E7 expression	Yes	No
p53 mutations	No	Yes
pRb downregulation	Yes	No
p16 overexpression	Yes	No

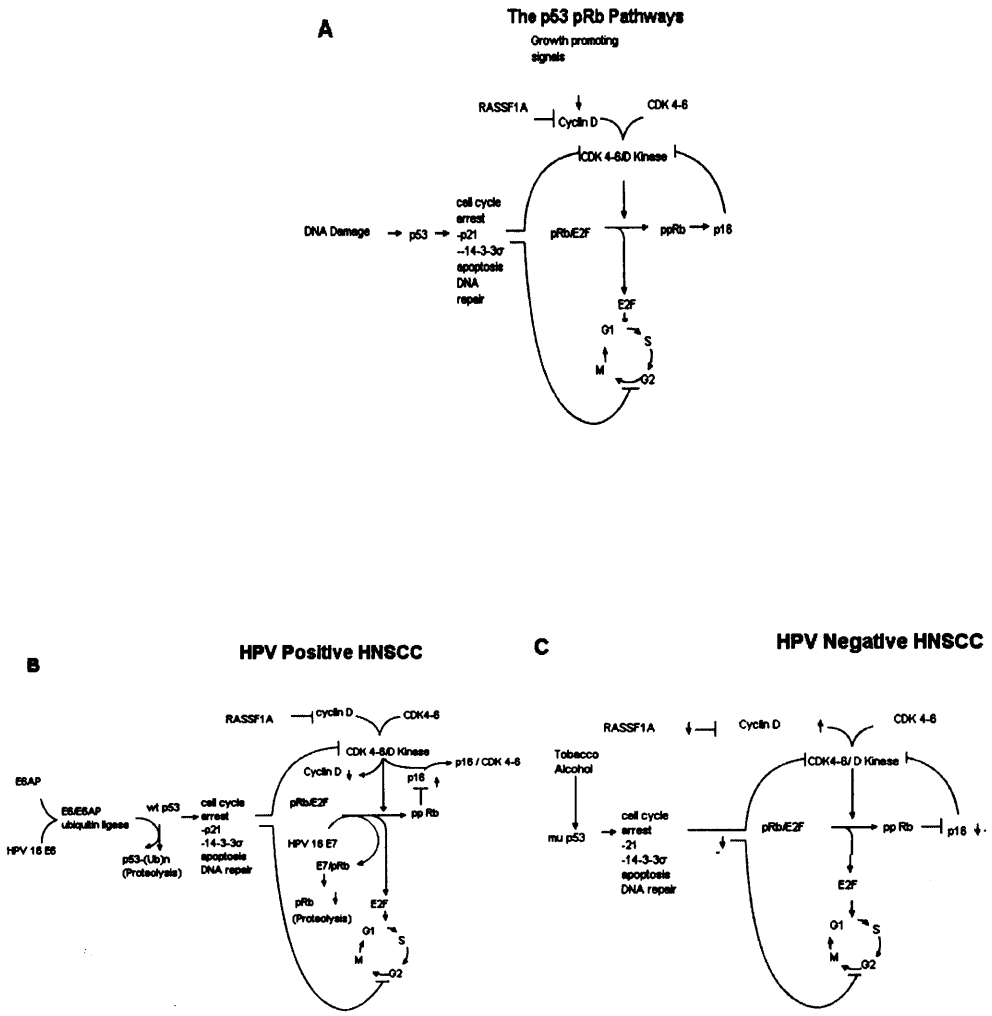


Figure 1.8 Different mechanisms in HPV-HNSCC and HPV-negative HNSCC. (A) The p53 and pRb pathways commonly altered in human cancers. (B) Molecular alterations in HPV-HNSCC reflect the activity of viral oncoproteins E6 and E7. HPV 16 E6 and E7 facilitate the proteolysis of p53 and pRb, respectively, and thereby disrupt control of cell cycle progression and DNA damage repair (C) Molecular alterations in HPV-negative HNSCC. It is characterized by tobacco-induced p53 mutations.
(Adapted from Gillison, 2004)

1.14.1 Humanpapilloma virus and tonsillar carcinoma

Epidemiological and molecular studies have suggested an aetiological role for HPV in tonsillar carcinomas, the detection rate being as high as 51% (Syrjanen, 2004). It has been observed that the palatine tonsils are the most likely tonsillar tissue to be infected with HPV among other tonsils. It has been suggested that the tonsillar tissue may be susceptible to infection by HPV that is present in oral and pharyngeal fluids. The HPV particles can easily access the basal cell layer due to the thining of the epithelium in some areas caused by the deep invagination of the tonsillar epithelial mucosa. In addition the local presence of the cytokines produced by lymph nodes tissues may positively influence HPV transcription and cellular transformation (Klussmann *et al.*, 2001; Syrjanen, 2004).

Recent studies demonstrated that patients with HPV-associated anogenital cancer had a 4.3 fold higher risk of tonsillar cancer than other patients with no HPV infection in the genital area (Frisch and Biggar, 1999; Syrjanen, 2004), which may suggest the involvement of oral sex habit as a route of transmission of the virus. High-risk type 16 was the most frequent type identified among tonsillar carcinoma samples, and the virus being mostly present in an episomal form. However, low risk HPV types 6/11 have also been detected in HPV-positive tonsillar carcinoma (Klussmann *et al.*, 2003; Syrjanen, 2004). Some studies have indicated that patients with HPV-16 positive tonsillar carcinoma tend to have a better long-term survival rate than those with HPV-negative disease, and thus the former seem to represent a distinct group of patients with tonsillar carcinoma (Syrjanen, 2004; Syrjanen, 2005).

1.14.2 HPV in salivary glands lesions

There is a little data concerning the association of HPV with salivary gland malignancies. One study examined the presence of CMV, HHV8, EBV and HPV in pleomorphic adenomas, acinic cell carcinoma, lymphomas of the parotid gland, squamous cell carcinoma of salivary gland, mucoepidermoid carcinomas, adenocarcinomas, adenoid cystic carcinomas, epithelial-myoepithelial carcinomas and myoepithelial carcinomas. The results showed no evidence of any association of HPV with salivary gland tumours (Atula *et al.*, 1998). Another study also failed to detect HPV in pleomorphic adenomas (Rommel *et al.*, 1991). Of note, a recent study had investigated the clinicopathological features of condyloma and condyloma like-lesion of the oral cavity and suggested that oral condyloma acuminatum may involve the excretory duct of minor salivary glands (Henley *et al.*, 2004).

1.14.3 HPV in HNSCC in young adults

An increasing interest has been observed for the possible role of HPV in OSCC in young adults (von Doersten *et al.*, 1995), which has a tremendous social implications in young populations since the disease is fatal. Most clinicians believe that young patients with HNSCC make up a distinct subset of HNSCC (usually males under 40 years of age and with little or no exposure to known risk factors such as heavy tobacco smoking and high alcohol intake), and more concern exists for the prognosis and treatment for these young patients. The association between tobacco and alcohol intake in OC of older patients has been documented. However, many studies have demonstrated a lack of involvement with the traditional habits of

smoking/alcohol consuming in such young group. Although, HPV has been proposed as a possible risk factor for the development of HNSCC, the role of HPV in young individuals with HNSCC has not been determined (Sisk *et al.*, 2000).

The relation between HPV positive HNSCC and prognosis/survival rate is not consistent. It has been suggested that patients with HPV infected HNSCC have a better prognosis compared with those without HPV infection (Haraf *et al.*, 1996; Gillison *et al.*, 2000; Smith *et al.*, 2004). Although, other previous studies have observed no differences between the two groups of patients (Riethdorf *et al.*, 1997; Pintos *et al.*, 1999). In deed, the prognosis of HPV positive patients was poorer than those with HPV negative HNSCC group (Clayman *et al.*, 1994). Interestingly, in one study when the results stratified for genders, HPV infected HNSCC males show a better prognosis than HPV negative males, but this was not the case among females (Ritchie *et al.*, 2003). Pertaining to recurrence, Gillison in 2002, have observed that patients with HPV positive HNSCC showed a 1.4 fold greater risk of recurrence when compared with HPV negative patients.

1.15 Other oral lesions associated with HPV

Histologically, it is known that the oral cavity is lined by a mucous membrane consists of a stratified squamous epithelium and lamina propria made up of dense connective tissue. The squamous epithelium of the gingiva, hard palate and the dorsum of the tongue are completely keratinized with a superficial horny layer, whereas in the lip, cheek, vestibular fornix, alveolar mucosa, floor of the mouth and soft palate, the epithelium is non-

keratinized. Thus, the histology of oral mucosa resembles that of the uterine cervix, other sites of the lower genital tract or skin, depending upon the anatomic site. In view of the similarities, both mucosal and cutaneous HPV types would be expected to infect the soft tissue of the mouth lining (Lakshmi *et al.*, 1993; Syrjanen, 2003). Considering that, in the genital region this virus is implicated in the development of cancer in the uterine cervix, it is possible that this link would also be present in relation to oral epithelial cancer. Therefore, it has become important to know the frequency of this virus in the oral cavity and its probable relationship with the presence of malignant neoplasm in the region. HPV infection is known to be associated with oral mucosal squamous cell papillomas (SCP), condyloma acuminatum (venereal warts), verruca vulgaris (oral warts), and focal epithelial hyperplasia (FEH) (Syrjanen, 2003).

1.15.1 Squamous cell papilloma (SCP)

Squamous cell papilloma is a relatively common benign tumour of the oral epithelium, it appears clinically as small finger-like projections, resulting in a lesion with a rough or cauliflower verrucous surface. Few researchers have examined the association of HPV and SCP by different diagnostic methods and the results varied considerably, mainly HPV-6, followed by HPV-11 are the types most commonly detected in oral squamous papilloma of the mouth (Greer and Goldman, 1974; Praetorius, 1997; Syrjanen, 2003).

1.15.2 Condyloma acuminatum (venereal warts)

Condyloma acuminatus is regarded as a sexually transmitted disease affecting the skin and mucous membranes of the anogenital tract. Oral

condyloma can be acquired not only by oral sex but also as a result of maternal transmission. In the genital tract, the term condyloma and papilloma were used separately, lately; both lesions have been called condylomas. This rule applied also with the oral lesions, since both condylomas and papilloma harbour the same HPV types found in the genital condylomas. Many studies showed the relation of HPV and condyloma acuminatum using different laboratory techniques, HPV types 2, 6, and 11 showed high presences in the samples up to 90%. One study used specific primers detected HPV 6/11, 16/18, 31/33 and 35 in lesional tissues (Zeuss *et al.*, 1991; Praetorius, 1997; Syrjanen, 2003).

1.15.3 Verruca vulgaris (oral wart)

Verruca vulgaris is the most common prevalent HPV lesion of the skin, but is also found in oral mucosa. The most common oral sites of these lesions are areas in which keratinization of the epithelium resembles that of the skin, i.e., lip, hard palate and gingiva. On clinical examination, verruca vulgaris is often indistinguishable from SCP and condyloma. To confirm the diagnosis, cutaneous HPV types should be identified in oral verruca. Human papillomaviruses types 2 and 57 are the mostly associated HPV types, although some studies have found HPV 6/11 in verruca vulgaris (Praetorius, 1997; Syrjanen, 2003).

1.15.4 Focal epithelial hyperplasia (FEH)

Focal epithelial hyperplasia is known as Heck's disease, manifests as multiple, soft, flat or rounded slightly elevated nodules. The lesions are asymptomatic, and may persist for several years, but they tend to regress

spontaneously, although that the lesions occasionally may recur. It was first described in Native American Indians, now it is also known to arise in some ethnic groups like the Eskimos (Jayasooriya *et al.*, 2004). It appears that HPV types 13 and 32 are strongly associated with FEH, these having been detected in 75%-100% of examined FEH specimens (Praetorius, 1997; Syrjanen, 2003; Jayasooriya *et al.*, 2004).

1.16 HPV in potentially malignant oral mucosa

It is known that the development of head and neck malignancies such as oral squamous cell carcinoma (OSCC), follow a multistep genetic progression from normal mucosa to invasive disease (Shin *et al.*, 2001). Early disease manifests histopathologically as dysplasia, which may have some of the genetic changes observed in OSCC. Such disease manifests clinically as a localized area of white and/or red patches described as leukoplakia or erythroplakia.

1.16.1 HPV in oral epithelial dysplasia

Oral epithelial dysplasia (OED) may be histologically graded as mild, moderate and severe, this being usually, but not always, a predictable gradient of malignant potential (Jaber *et al.*, 2003; Sudbo *et al.*, 2004). Oral epithelial dysplasia (OED) is thought to be principally caused by prolonged high tobacco usage and/or alcohol consumption (Jaber *et al.*, 1999). In addition OED can arise with lichen planus (Epstein *et al.*, 2003; Dey, 2004) and in areas adjacent to OSCC (Chang *et al.*, 1990). Thus, OSCC may be preceded by epithelial changes similar to those of CIN. Thus, it would not be

unexpected for HPV to be detected in OED. In deed, HPV-DNA has been detected in 0-85% of lesions with clinical and/or histopathological features of likely OED (Gassenmaier and Hornstein, 1988; Bouda *et al.*, 2000; Sugiyama *et al.*, 2003; Ha and Califano, 2004). If HPV is of importance in the aetiology of oral squamous cell carcinoma, it would be expected to be present in oral epithelial dysplasia (Table 1.6).

However, the results of some of the previous studies must be considered carefully as they did not clearly detail the presence and/or severity of the OED in the examined lesions. In addition, a plethora of different methods of detection of HPV has been employed in these studies of OED and other potentially malignant diseases of oral mucosa and OSCC. Nevertheless, high risk HPV genotypes particularly HPV-16 or 18 were typically present in OED (or OED-like) lesions that carried HPV (Ha and Califano, 2004). Sugiyama and colleagues in 2003, suggested that the increased carriage of HPV in potentially malignant lesions does raise the possibility that this virus may act as an initiator of epithelial proliferation or play a role in the early stage of oral carcinogenesis.

Other diseases considered potentially malignant have also been found to be infected with HPV. These include oral lichen planus (OLP) (15.4% - 87.5%) (Kashima *et al.*, 1990; Jontell *et al.*, 1990; Wen *et al.*, 1997b; Sand *et al.*, 2000; Ostwald *et al.*, 2003; Lazzari *et al.*, 2004), proliferative verrucous leukoplakia (PVL) (10% - 89%) (Palefsky *et al.*, 1995; Gopalakrishnan *et al.*, 1997; Fettig *et al.*, 2000; Campisi *et al.*, 2004).

1.16.2 HPV in proliferative verrucous leukoplakia

Proliferative verrucous leukoplakia is known as a very aggressive form of oral leukoplakia with high morbidity and mortality rates due to its high potential for malignant transformation. Hansen *et al* first described PVL in 1985, and it is characterized histopathologically by the presence of multi-focal hyperkeratotic lesions, which can easily be misinterpreted as a form of simple hyperkeratosis. In the recent years, PVL is considered as a lesion with an uncharacteristic entity with high risk of malignant transformation. In deed, the transformation rate in PVL is reported in up to 86.7% of the cases (Hansen *et al.*, 1985; Silverman S Jr and Gorsky, 1997). The clinical course of PVL is gradual but progressive, begins as hyperkeratotic plaques that slowly progress into exophytic verrucous plaques. Focal areas of erythroplakia are common as a late manifestation of the disease. Patients affected with PVL are often middle-aged non-smokers/alcohol drinker females (Batsakis *et al.*, 1999; Greer *et al.*, 1999). The aetiology of PVL remains elusive. Smoking and the presence of *Candida* are no longer thought to have any influence on the occurrence and progression of PVL (Vigliante *et al.*, 2003). In the literature, there are few studies investigating the association between PVL and HPV, detection rates ranging from 10-25% (Palefsky *et al.*, 1995) to only in 89% of the cases (Gopalakrishnan *et al.*, 1997; Fettig *et al.*, 2000). In PVL, a non-specific and likely aetiological role of HPV infection was eagerly suspected due to the clinical and histological warty features of PVL-related lesions. The role of HPV remains speculative regarding the significance of its presence and possible regulatory influence on PVL-occurrence and progression (Silverman S Jr and Gorsky, 1997;

Vigliante *et al.*, 2003). In fact, Palefsky and co-workers in 1995 reported that HPV-16 plays a fundamental role in the pathogenesis of PVL-associated oral epithelial lesions, where HPV type 16 is known to be highly associated with cervical cancer. However, a recent study has established that the frequency of HPV infection of PVL is not any higher than that of non-proliferative supposed potentially malignant leukoplakias (Palefsky *et al.*, 1995; Campisi *et al.*, 2004).

1.16.3 HPV in oral lichen planus

Oral lichen planus (OLP) is a common chronic inflammatory disorder present in about 0.1 - 4% of the population and usually appears in patients over 50 years of age (Scully and el-Kom, 1985). Clinically, OLP presented in a variety of clinical types (i.e. reticular, popular, plaque-like, atrophic/erosive, ulcerative, and bullous), characterized by multiple lace-like lesions, erosions, and ulcerations, affecting mostly the buccal mucosa, followed by the tongue and gingiva. Histologically, OLP is characterized by epithelial atrophy, basal cell destruction, and a dense band-like infiltrate of mononuclear inflammatory cells in the superficial corium (Andreasen, 1968; Eisen, 1993). The World Health Organization (WHO) categorized OLP as a premalignant condition, particularly if there is evidence of dysplasia. However, the malignant transformation of OLP remains a controversial matter. Follow-up studies showed that the rate of OLP transforming to OC ranges between <1-5.3 (Mattsson *et al.*, 2002). Many researchers in recent years have suggested the viral infection as a possible factor or co-factor in the pathogenesis of OLP, of those, were human herpes viruses and HPV (Kashima *et al.*, 1990;

Jontell *et al.*, 1990; Gonzalez-Moles *et al.*, 1994; Ghodratnama *et al.*, 1997; Sun *et al.*, 1998). The involvement of HPV in the aetiology of OLP has been proposed by some authors (Miller and White, 1996), yet the exact involvement, and role of such viruses still need further considerations.

Table 1.6 HPV prevalence in oral cavity pre-malignant lesions

Study	Mode of Detection	HPV+*	%	Lesion Type
Maitland *et al.*, 1987	SB using HPV 16 probe	16/ 21	28.6	Dysplasia keratosis hyperplasia lichen planus
Gassenmaier and Hornstein, 1988	ISH	19/103	18.4	Dysplasia
Syrjanen *et al.*, 1988a	ISH 6, 11, 13, 16, 18, 30	6/ 22	27.3	Dysplasia CIS
Greer *et al.*, 1990	ISH 6, 11, 16, 18, 31, 33, 35	5/190	2.6	Leukoplakia dysplasia
Shroyer and Greer, 1991	E6 HPV-16 PCR/ISH	7/ 48	14.6	Dysplasia hyperplasia keratosis
Zeuss *et al.*, 1991	ISH 6/11, 16/18, 31/33/35	0/ 20	0	Dysplasia CIS
Holladay and Gerald, 1993	E1 PCR	13/ 45	28.9	CIS dysplasia inflammation hyperplasia
Fouret *et al.*, 1995	E6 consensus PCR	0/ 3	0	Dysplasia
Mao *et al.*, 1996	L1 consensus and E6/7 PCR	8/ 23	34.8	Dysplasia CIS
Nielsen *et al.*, 1996	ISH/ HPV 16 PCR, SB PCR	20/ 49	40.8	Dyplasia leukoplakias
Wen *et al.*, 1997	E6 HPV 16/18 PCR	5/ 17	29.4	Papilloma leukoplakias lichen planus
D'Costa *et al.*, 1998	L1 consensus PCR	27/ 80	33.8	Leukoplakias lichen planus submucous fibrosis melanoplakia
Elamin *et al.*, 1998	Nested L1 PCR	4/ 12	33.3	Dysplasia
Matzow *et al.*, 1998	Consensus PCR	1/ 5	20	CIS hyperplasia
Bouda *et al.*, 2000	Nested consensus PCR	29/ 34	85.2	Hyperplasia dysplasia
Sand *et al.*, 2000	L1 consensus type-specific	8/ 29	27.6	Lichen planus leukoplakias
Ha *et al.*, 2002	Quantitative PCR	1/102	1.0	Dysplasia CIS

ISH= In situ hybridization, PCR= Polymerase chain reaction, SB=Southern blotting
(Adapted from Ha and Califano 2004)

1.17 HPV in immunosuppressed and HIV-infected individuals

Some individuals with HIV-related or iatrogenic immunosuppression seem to be at risk of human papillomavirus-associated anogenital cancers (Palefsky and Holly, 2003). Many studies have demonstrated that patients with HIV infection and AIDS are at increased risk of developing specific types of cancers, particularly Kaposi's sarcoma, non-Hodgkin's lymphoma and cervical or perioral SCC (Biggar *et al.*, 1996; Beral and Newton, 1998; Frisch *et al.*, 2000). Most cancers in patients with AIDS are related to oncogenic virus infections, such as EBV, KS and HPV (Melbye *et al.*, 1994;

teenyi-Agaba, 1995; Goedert *et al.*, 1998; Frisch *et al.*, 2000; Aoki and Tosato, 2004).

The relationship between HIV and cervical cancer is more complicated than that of HIV and KS; there have been some suggested limitations to the role of the HIV in cervical neoplasia and malignancy. Current data suggest that immunosuppression is not associated with the progression of dysplastic tissues to cancer. Although, it is strongly associated with the early stages of dysplasia, the immune response may play an important role in controlling HPV replication and development of early disease such as CIN I. In contrast, progression from high-grade lesion to cancer may be affected by cellular genetic changes (Frisch *et al.*, 2000; Palefsky and Holly, 2003; Palefsky, 2003; Aoki and Tosato, 2004). It has been suggested that HPV-persistence in HIV-infected women is linked to a reduction in HLA class II molecules and to a greater number of immature Langerhan's cells within the cervix (Goncalves *et al.*, 2004; Moodley, 2005). From a molecular point of view, it has been suggested that HIV-associated cancers are likely to progress via microsatellite instability pathways, while pathways that involve loss of hetrozygosity of tumour suppressor genes may cause HIV-negative cancers (Clarke and Chetty, 2002; Moodley, 2005). In addition, men infected with HIV and having low CD4 counts showed an increased incidence of HPV infection, increased HPV viral load, more persistent detection of HPV and are more likely to have multiple types of HPV compared with HIV negative men (Palefsky *et al.*, 1998; Hagensee, 2000). However, it has been observed that organ transplant recipients are at increased risk of HPV lesions in the oral mucosa, skin and anogenital areas (Blessing *et al.*, 1990). In

immunosuppressed patients, oral papillomas and warts usually have an atypical morphology, and the HPV types detected are often unusual, e.g. HPV type 7, followed by types 13, 18 and 32 (Greenspan *et al.*, 1988). To demonstrate how unusual some of theses findings are, type 7 was originally isolated from peculiar type of cutaneous wart, frequently present on the hands of butchers (Greenspan *et al.*, 1988; de Villiers, 1989; Syrjanen, 2003).

The effect of HAART on the incidence and prevalence of HPV infection, HPV viral load and CIN in HIV-positive women remains extremely conflicting. Some data suggest that the widespread of HAART has not lead to reduced prevalence of genital HPV infection on HIV-positive women (Palefsky, 2003), while in a recent study, it has been suggested that HAART can enhance the regression of CIN (Prins *et al.*, 2005). Furthermore, the widespread administration of HAART showed a profound impact on the incidence of HIV-associated oral opportunistic infections. The incidences of oral thrush and oral hairy leukoplakia have significantly decreased (Greenspan *et al.*, 2001). Interestingly, in contrast to these infections, an increase in incidence of oral papillomas (warts) have been observed since the advent of HAART (Leigh, 2000). In fact, a significant association between the rise in warts and HAART have been reported (Greenspan *et al.*, 2001; King *et al.*, 2002). The role of HAART on the rate of oral HPV is not clear yet, but suggestions that have been proposed include that HAART associated increase in CD4+ levels does not reflect HPV-specific immune reconstitution, also HAART may lead to immune reconstitution related to other pathogens, but has no effect on HPV-specific immunity (Palefsky and Holly, 2003)

1.18 Summary of HPV and oral diseases

In the past two decades, there has been an increasing interest in the association of HPV with different oral diseases, due to some degree to its likely role in the pathogenesis of malignant tumours in the cervical and ano-genital areas. The morphological similarities between oral and cervical mucosa would suggest that HPV could play a role in oral mucosal oncogenesis. Epidemiological and experimental evidence has implicated oncogenic HPV in the development of a subset of HNSCC (most commonly high-risk types 16 and 18). Nevertheless, the frequency of HPV in oral mucosal lesions varies with the geographic locales and the methods of assays employed.

1.19 Human herpesviruses

1. 19.1 General features of herpesviruses

Herpesviridae are the family of enveloped, double-stranded DNA. The viruses replicate in the nucleus of a wide range of humans and animals. There are 80 known herpesviruses, and at least eight of them are known to cause infection in humans. These include herpes simplex virus (HSV) 1 and 2, varcilla zoster virus (VZV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpes virus 6(HHV-6), human herpes virus 7(HHV-7), and human herpes virus 8 (HHV-8) (Table 1.7). They are transmitted from host to host by direct contact, causing a primary infection and then remain latent within the nuclei of specific cells. The site of latency differs among the herpesviruses. Herpesviruses (1, 2, and VZV) are known to remain latent in the sensory nerve ganglia, CMV remains latent in lymphocytes and possibly in salivary gland tissues, EBV remains latent in B lymphocytes and salivary gland tissues, herpesviruses (6 and 7) remain latent in CD4 lymphocytes and HHV-8 remains latent; although the exact site of latency is still not known (Stoopler and Greenberg, 2003).

Table 1.7 The Herpesviruses family is divided into 3 subfamilies:

Alphherpesvirinae	*Simplexvirus*	*Human herpesvirus* 1 (HSV 1, 2)
	Varicellovirus	*Human herpesvirus* 3 (VZV)
Betaherpesvirinae	*Cytomegalovirus*	*Human herpesvirus* 5 (HCMV)
	Roseolovirus	*Human herpesvirus* 6 (HHV 6, 7)
Gammherpesvirinae	*Lymphocryptovirus*	Human *herpesvirus* 4 (EBV)
	Rhadinovirus	*Simian herpesvirus* 2 (HHV 8)

They share four characteristics biological properties:

- Synthesis of viral DNA and assembly of their capsid occur in the nucleus of infected cell.
- They encode their own enzymes involved in nucleic acid metabolism, DNA synthesis and protein processing.
- Production of their infectious viral particles is usually accompanied by destruction of the infected cell.
- Latent infection occurs which persist for the life of their natural hosts. The latent viral genomes usually take form of circular episomes, with only a small subset viral genes being expressed.

1.19.2 Human herpesviruses 1 and 2

Human herpesviruses 1 and 2 are the two major types of herpes simplex viruses and are the causative agents in most common intraoral herpesviruses infections. Diagnosis is based on the antibodies formed against each type of virus or by DNA analysis (Greenberg MS, 2003). Classically, HSV 1 causes a majority of oral and pharyngeal HSV infections, meningoencephalitis and dermatitis above the waist; while HSV 2 is implicated in most genital and anal infection (Stoopler, 2005). It has been suggested that the sexual practices may be a cause of primary or secondary infection of HSV 1 and 2 in both oral and genital areas (Stoopler and Greenberg, 2003)

1.19.2.1 Primary herpes simplex virus infection

It has been demonstrated that the incidence of primary infections with HSV 1 increases after 6 months of age due to the loss of anti-HSV antibodies acquired from the mother during gestation. The incidence reaches a peak between 2-3 years of age, but the infection may occur in the adolescents and adults with occasional cases to occur in patients over 60 years of age. While it has been postulated that the primary HSV 2 infection does not increase until sexual activity begins (Stoopler and Greenberg, 2003). The majority of primary HSV 1 and 2 infections are sub-clinical or may cause a pharyngitis making it difficult to distinguish it from upper respiratory tract viral infections. The symptomatic infections usually preceded or accompanied by generalized symptoms including fever, headache, malaise, nausea, and vomiting. Lymphoadenopathy may be evident in occasions. In the oral cavity, vesicles and ulcers appear on the oral mucosa, and usually after 24 to 28 hours after the prodromal symptoms, a generalized acute marginal gingivitis usually occur. In healthy children, primary HSV is usually a self-limiting disease with fever disappearing in 3-4 days and the oral lesions healing within 7-10 days (Stoopler, 2005).

1.19.2.2 Recurrent herpes simplex virus infection

Following the resolution of a primary HSV infection, the virus migrates to the trigeminal nerve ganglion, where it is capable to remain in a latent state. Reactivation of the virus may follow an exposure to cold, exposure to sunlight, stress, trauma or immunosuppression disease. Recurrent herpes labialis (RHL) is the most common form of recurrent oral HSV infection

appear on the border of the lip and is commonly referred to as cold sore or a fever blister (Stoopler, 2005).

1.19.3 Varcilla zoster virus (VZV)

Varicella zoster virus causes both primary (chickenpox) and recurrent infection (shingles) and remains latent in neurons present in sensory ganglia (Greenberg MS, 2003).

1.19.3.1 Varicella

Chickenpox is a benign disease of children, spreads by direct contact with either the skin lesions or nasopharyngeal secretions of an infected patient. The incubation period is 10 to 21 days, and the stage is highly infectious for 1 week after symptoms start. One of the major complications is encephalitis causing high rate of mortality among adults. Other complications include pneumonitis and Reye's syndrome (Stoopler, 2005). Skin lesions of chickenpox are characterized by maculopapular lesions that are intensely pruritic. These lesions rapidly progress to fluid-filled vesicles on an erythematous base. Oral lesions may appear in vesicle/ulcer form, which is not significantly an important symptom (Greenberg MS, 2003).

1.19.3.2 Herpes zoster (HZ)

Following the primary infection, VZV becomes latent in dorsal root of cranial nerve ganglia. Only in a small range of population, the virus becomes reactivated causing HZ. When HZ involves the trigeminal nerve ganglion, the first division (ophthalmic or V1) is most commonly involved. Pain is the main

initial symptom of HZ, also tenderness and parasthesia along the course of the affected nerve. Unilateral vesicles appear 3 to 5 days later, when the geniculate ganglion of the facial nerve is infected, typical clinical signs and symptoms appear include unilateral vesicles of the external ear and oral mucosa as well as unilateral facial paralysis (Stoopler, 2005).

1.19.4 Human herpesvirus 6

Human herpesvirus was first discovered in 1986 (Salahuddin *et al.*, 1986). It is tropic mainly for CD+4 T cells, however, the replication can also occur in CD+8 cells, macrophages and epithelial cells. CD46 has been recently identified as a cellular receptor for HHV-6 (De Araujo *et al.*, 2002). The virus is ubiquitous in the population with greater than 90% seropositivity in adults, and has a worldwide distribution. The virus is transmitted via saliva, and usually occurs during the first 2 years of life (Stoeckle, 2000). It has been postulated that immunocompromised patients, in particular, solid organ transplant, are at greater risk of HHV-6 disease (Emery, 2001).

1.19.5 Human herpesvirus 7

Human herpesvirus 7 was first isolated in 1990 (Frenkel *et al.*, 1990) from CD+4 cells of healthy adults. It has been found that HHV-6 and HHV-7 are closely related by DNA analysis. Human herpesvirus 7 has been isolated from saliva of approximately 90% of healthy individuals worldwide. No clear association between HHV-7 and any specific clinical disorder has been reported, although, it has been associated with febrile illness in children, exanthema subitum and pityriasis rosea (De Araujo *et al.*, 2002).

1.20 Human cytomegalovirus

1.20.1 Virology

Human cytomegalovirus (HHV-5) is a β human herpesvirus; it is the largest known human herpesvirus, with a genome of about 230 kb. The virus has double strand linear DNA, enveloped by a proteinaceous matrix (the tegument), which is surrounded by a lipid bilayer that contains viral glycoproteins (Figure 1.9). Human cytomegalovirus can be transmitted via saliva, sexual contact, placental transfer, breastfeeding, blood transfusion, solid organ transplantation or haematopoietic stem cell transplantation. It has been demonstrated that the acquisition of the virus rises progressively from an early age. The overall seroprevalence of CMV in developed countries has been found to be between 30 and 70% (Pass, 1985). However, homosexual men, individuals in low socioeconomic groups and residents of developing countries have seroprevalence rates that can exceed 90% (Sohn *et al.*, 1991; Gandhi and Khanna, 2004).

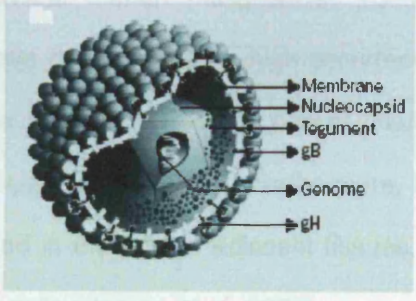


Figure 1.9 Schematic diagram of Cytomegalovirus
Adapted from: http://www.biografix.de/

It has been suggested that after the primary infection, cytomegalovirus will establish a lifelong latency or persistence within the human host in which the principal reservoirs are fibroblasts, myeloid and endothelial cells (Mocarski, Jr., 2002; Rowshani et al., 2005). However, it is not clear which cell types can harbour the virus and support a productive (lytic) infection, nor it is clear whether leukocytes behave as specific carriers of the virus during a systemic infection or represent a reservoir of replicating virus, or a possible site of latency. Although primary infections or reactivation of CMV usually remain either subclinical or self-limiting in immunocompetent individuals, this virus is a major cause of morbidity and mortality in immunocompromised patients, especially in organ transplants recipients, patients receiving immunosuppressive therapy and in those with AIDS (Kano and Shiohara, 2000).

1.20.2 CMV and malignancies

Cytomegalovirus DNA, RNA and/or proteins, have been detected in some tumour tissues, and CMV infection has been implicated in the pathogenesis of several human malignancies (Cinatl, Jr. *et al.*, 2004). Recently, studies have demonstrated a high occurrence of CMV in prostate carcinoma (Samanta *et al.*, 2003), colon cancer (Harkins *et al.*, 2002) and malignant glioma (Cobbs *et al.*, 2002). Furthermore, it has been found that no virus was detected in the normal adjacent tissues, indicating a selective replication in tumour cells (Hoever *et al.*, 2005).

It has been suggested that the multiple oncogenic activities of CMV that infleuence cell cycle progression, angiogenesis, cell invasion and

immune evasion in cells may account for such associations with malignancy (Doniger et al., 1999; Castillo and Kowalik, 2002; Samanta et al., 2003). CMV has the ability to interfere with many cellular pathways, which influence the malignant behaviour of tumour cells, namely apoptosis, adhesion, invasion and angiogenesis. It can also dysregulate many multiple cellular pathways and signalling mechanisms involved in malignancy, including targeting Rb and p53 that result in promoting of cell cycle progression, induction of DNA mutations and blocking of apoptotic pathways (Harkins et al., 2002; Samanta et al., 2003). This is similar to the activities of oncoproteins in small DNA tumour viruses such as human papillomavirus or adenoviruses. It has been suggested that persistence in tumour cells is essential for CMV to fully express its oncomodulatory effects (Cinatl, Jr. *et al.*, 2004; Hoever *et al.*, 2005).

In a recent study of colorectal cancer, investigators observed that the preferential replication of CMV is in dysplastic epithelial cells, these cells are known to harbour replicative virus in immunocompromised patients, suggesting that dysplastic epithelial cells with impaired cell-cycle control mechanisms may provide a unique reservoir for CMV persistence with no effect on the overall tumour phenotype. Alternatively, long-term persistent CMV infection and expression in dysplastic epithelial cells could be important in the promotion of oncogenic action directly implicated in malignant progression (Harkins *et al.*, 2002).

Although CMV is well known to interfere with many cellular pathways which influence the malignant behaviour of tumour cells (Cinatl, Jr. *et al.*, 2004; Hoever *et al.*, 2005), its relation with oral cancer has not been yet

defined. A study investigating the involvement of viral and chemical factors with oral cancer in a Taiwanese study group demonstrated that CMV was not directly involved in the oncogenic process (Yang *et al.*, 2004).

1.20.3 CMV in salivary gland tissue

The presence of CMV-DNA in salivary gland benign and malignant tumours has been investigated using ISH and PCR techniques. However, it appears that CMV plays no role in the aetiology of salivary glands neoplasms. (Laane et al., 2002; Rivera et al., 2003; Yen et al., 2004).

1.20.4 CMV in oral lesions

Cytomegalovirus is commonly found in periodontal abscesses as well as in necrotic pulps and periapical lesions (Sabeti and Slots, 2004; Santangelo et al., 2004; Saygun et al., 2004), and has been proposed as a putative pathogen in these lesions causing pathosis either by inducing immunosuppression with a subsequent risk of aggressive bacterial infection or by infecting periodontal cells directly. Alternatively, CMV may give rise to periapical disease by inducing cytokines and chemokine release from inflammatory and non-inflammatory host cells (Mogensen and Paludan, 2001; Slots J, 2002; Sabeti et al., 2003; Sabeti and Slots, 2004).

1.21 Epstein-Barr virus

1.21.1 Virology

Epstein-Barr virus (EBV) or human herpesvirus 4 (HHV-4) is a γ-herpesvirus which preferentially infects B-lymphocytes, epithelial cells, T cells, natural killer (NK) cells and smooth muscle cells. It infects the vast majority of adults worldwide, establishing both non-productive (latent) and productive (lytic) infections (Iwatsuki *et al.*, 2004).

The EBV genome is a linear double-stranded DNA of 172 kbp and was the first herpesvirus genome to be completely sequenced (Baer *et al.*, 1984) (Figure 1.10). It is able to immortalize B cells both *in-vitro* and *in-vivo*. Primary EBV syndromes include infectious mononucleosis, chronic EBV infection and X-linked lymphoproliferative syndrome (fatal infectious mononucleosis) (Goldenberg *et al.*, 2001).

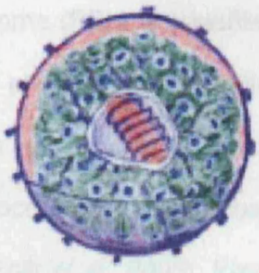


Figure 1.10 Schematic diagram of Epstein Barr-virus
Adapted from
http://www.brown.edu/Courses/Bio_160/Projects2000/Herpes/EBV/Epstein-Barr.html

1.21.2 EBV and malignancies

Epstien-Barr virus has a variety of mechanisms that promote immortalization of epithelial cells (as in nasopharyngeal carcinoma and oral

cancer), B-lymphocytes (as in Burkitt's lymphoma and plasmablastic lymphoma), T-lymphocytes (as in NK T-cell lymphoma) and Hodgkin's disease. Epstein-Barr virus is a ubiquitous human herpes virus that can cause a lifelong persistent infection of B cells in more than 90% of the adult population. In addition it is known to be a B-lymphotropic virus and has the ability to transform B cells *in vitro*. (Goldenberg *et al.*, 2001; Pagano *et al.*, 2004; Parkin, 2006).

Burkitt's lymphoma (BL) is a high-grade B cell lymphoma that occurs in children and young adults worldwide. Approximately 95-100% of African BL tumours contain EBV-DNA, whilst only 20-30% of similar tumours in persons resident in Europe and the US are EBV-associated. In the Middle East, North Africa and South America, 51-91% of BL tumours are associated with EBV. These findings suggest that EBV may not itself be sufficient nor essential for the genesis of this lymphoma (Goldenberg *et al.*, 2004; Parkin, 2006).

Plasmablastic lymphoma (PBL) is classified by the WHO as a unique NHL type, characterized by plasma cell differentiation and an immunoblastic cellular morphology (Vega *et al.*, 2005). It mainly affects HIV-infected individuals, although it has occasionally been observed in immunocompetent patients (Lin *et al.*, 2004; Folk *et al.*, 2006). Plasmablastic lymphoma has a strong affinity for the oral cavity, stomach, lung, cervical lymph nodes, anorectal region and paranasal sinuses (Folk *et al.*, 2006). The most common oral sites for plasmablastic lymphoma are the gingivae, palate and floor of mouth (Delecluse *et al.*, 1997; Porter *et al.*, 1999; Borrero *et al.*, 2002).

Delecluse and co-researchers were the first to describe the strong association between EBV and plasmablastic lymphoma, detecting EBV in 9 out of 15 cases of PBL using *in situ* hybridization (Delecluse *et al.*, 1997). Researchers in recent studies have detected EBV-DNA in most of the examined PBL lesions, but none of the examined positive cases demonstrates EBV-LMP-1 expression. It has thus been suggested that EBV may play a role in the pathogenesis of a majority of PBL but not necessarily be the major causative agent (Gaidano *et al.*, 2002; Folk *et al.*, 2006).

Recently, EBV has also been implicated in the pathogenesis of NK T-cell lymphomas and has been identified within the NK-T tumour cells (Heslop, 2005).

As previously mentioned, EBV can infect epithelial cells causing malignancies, most commonly nasopharyngeal carcinoma. Nasopharyngeal carcinoma (NCP) is a relatively rare disease worldwide, having a unique geographic distribution. The highest incidence has been reported in Chinese populations although NCP can occur in other ethnicities (for example South East Asians and Eskimos).

It has been suggested that EBV could possibly play a role in the pathogenesis of oral squamous cell carcinoma as it has the ability to infect epithelial cells. However, the association of EBV and head and neck cancer remains controversial (Cruz *et al.*, 1997; Cruz *et al.*, 2000; Goldenberg *et al.*, 2001; Sand *et al.*, 2002; Tsang *et al.*, 2003; Goldenberg *et al.*, 2004; Iamaroon *et al.*, 2004). Despite the well-established influence of EBV on human B-lymphocytes, the influence of EBV in oral squamous cell carcinomas (OSCC) remains unclear. There has been several reports of

EBV-DNA occurring in both non-malignant and malignant oral squamous epithelial tissues (Talacko *et al.*, 1991; Mao and Smith, 1993; D'Costa *et al.*, 1998).

Recent studies in Japan have suggested a strong correlation between EBV and HNSCC in the Okinawa region, where the OSCC incidence rate is 1.5 times higher than in the mainland, thus supporting the hypothesis that geographical variations and racial factors may influence the development of EBV-associated OSCC (Tsuhako *et al.*, 2000; Higa *et al.*, 2003). In the literature, a possible relationship between EBV infection and the degree of differentiation of OSCC has been suggested (Kobayashi *et al.*, 1999; Shimakage *et al.*, 2002; Sand *et al.*, 2002). However, some studies report no evidence of an association between EBV and OSCC (Cruz *et al.*, 2000; Tsang *et al.*, 2003; Yang *et al.*, 2004; Goldenberg *et al.*, 2004). One study concluded that there was no evidence to support a clonal association between EBV with the neoplastic cells of OSCC, and that the presence of EBV in oral cancer samples may simply represent increased shedding of the virus in the oral cavity caused by malignancy-associated immunosuppression (Cruz *et al.*, 1997). However, EBV-derived latent membrane protein 1 (LMP-1) gene was detected only in the tumour cells of malignancy located in the nasopharynx, implying that EBV may play an insignificant role in the tumourogenesis of carcinomas arising from other locations in the head and neck region (Shimakage *et al.*, 2002; Tsang *et al.*, 2003). In addition, it has been suggested that the low quantities of EBV detected in a small group of HNSCC may originally have been from rare lymphoid or epithelial cells close to the primary HNSCC (Liu *et al.*, 2002).

It has been suggested that the presence of EBV in OSCC is not necessarily an indicator that the virus plays an oncogenic role. It has been suggested that as EBV is prevalent in the general population, it may be possible that neoplastic transformation took place in a cell harbouring EBV, the virus having no role in the process (Goldenberg *et al.*, 2001; Chan *et al.*, 2004). Similarly, it has been suggested that, EBV is unlikely considered to be a passenger virus as it has a strong capacity to stimulate cell growth and may play a role in the initiation stages rather than the maintenance stage of tumourogenesis(Griffin, 2000).

In general, it has been demonstrated that EBV-related malignancies may arise after years of viral dormancy being accompanied or triggered by viral reactivation (Harkins *et al.*, 2002). Epstein-Barr virus encoded latent membrane proteins-1 (LMP-1), have been described as the main transforming protein of EBV. It functions as a classic oncogene in rodent-fibroblast transformation assays and is essential for EBV-induced B cell transformation *in vitro*. When LMP-1 is expressed in cells it results in the induction of cell-surface adhesion molecules and activation antigens, and upregulation of anti-apoptotic proteins (Eliopoulos and Young, 2001). Furthermore it has been shown to function as a constitutively activated member of the tumour necrosis factor (TNF) receptor, leading to activation of several signalling pathways. EBV protein also activates several downstream signalling pathways that contribute to many phenotypic consequences of LMP-1 expression, including the induction of various genes that encode anti-apoptotic proteins and cytokines (Young and Rickinson, 2004). In addition, the EBV nuclear antigen (EBVNA-LP) has been shown to interfere with wild

type p53 and pRb functions, resulting in dysregulation of the cell cycle (Goldenberg *et al.*, 2001).

1.21.3 EBV in salivary gland lesions

Although it is known that EBV can be transmitted via saliva, the cellular source of the virus is still unclear. Some have suggested that the oral epithelium is a putative reservoir as well as the salivary glands. Various studies have observed no relation between EBV and salivary glands lesions (Karja *et al.*, 1997; Laane *et al.*, 2002), while others suggested EBV to be associated with salivary gland tumours, including a lymphoepithelioma-like carcinoma (Tsai *et al.*, 1996), undifferentiated carcinoma of the salivary gland (Wen *et al.*, 1997a), lymphoma of the parotid gland and pleomorphic adenoma arising in the nasal cavity (Atula *et al.*, 1998). The association of lymphoepithelial carcinoma of the salivary glands has been reported to be limited to Eskimos and Southern Chinese populations (Saemundsen *et al.*, 1982; Goldenberg *et al.*, 2001). Furthermore, it has been occassionaly reported in Arabs in Saudi Arabia (Abdulla and Mian, 1996) and Greek Caucasians (Kotsianti *et al.*, 1996) .

A recent study detected the presence of EBV-DNA in the labial minor salivary gland of HIV infected patients who had diffuse infiltrative lymphocytosis syndrome (DILS), suggesting the involvement of EBV in this unusal disease (Rivera *et al.*, 2003).

1.21.4 EBV in oral lesions

Epstein-Barr virus gives rise to infection monoclueosis, which can give rise to short-term oral manifestations such as palatal peteachia and

superficial oral ulcerations (Ebell, 2004). However by far, the most common and significant chronic EBV-associated disease of the mouth is hairy oral leukoplakia (OHL). Oral hairy leukoplakia arises in almost all groups of long-term immunocopmromised individuals, but particularly those with untreated HIV disease. It is clinically characterized by asymptomatic bilateral (often-elevated) white patches of the lateral borders and dorsum of tongue. EBV-DNA is abundant in OHL tissue (Hille *et al.*, 2002; Walling *et al.*, 2004; Komatsu *et al.*, 2005), yet, OHL has no known malignant potential, thus perhaps suggesting that EBV is unlikely to play a strong role in the development of OSCC in HIV cases (Scully and Porter, 2000).

1.22 Human Herpesvirus-8

1.22.1 Virology

Chang *et al* first identified human herpesvirus-8 (HHV-8) or Kaposi's sarcoma herpesvirus (KSHV) in 1994 based upon DNA sequences detected in tissues from patients with AIDS-KS.. Human herpesvirus-8 DNA or RNA has been localised by PCR *in-situ* hybridisation and *in-situ* hybridisation to spindle cells, endothelial cells and monocytes in nodular KS. It has been postulated that HHV-8 infected T cells and monocytes may be responsible for the circulation of HHV-8, accounting for the multicentric nature of some KS lesions (Hengge *et al.*, 2002b). Several clinical manifestations of KS have been distinguished including classic KS, endemic African KS, iatrogenic immunosuppressive KS and AIDS-related KS (Schulz, 2000; Hengge *et al.*, 2002a).

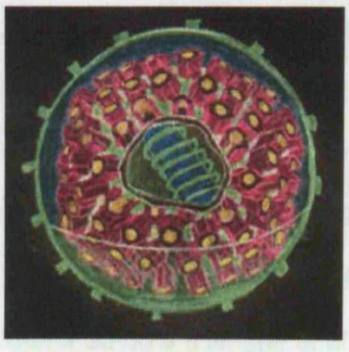


Figure 1.11 Schematic diagram of Human Herpesvirus-8
Adapted from: http://www.herpes.ru/her/pat/hhv8/

It has been demonstrated that HHV-8 has a tropism for lymphocytes, endothelial cell, keratinocytes and possibly marrow stromal cells. Its protein capsid structure is surrounded by an amorphous tegument and a lipid bilayer

for a general size of 120-150 nm. The HHV-8 genome is ~ 165 kb long and similar to that of other γ -herpesviruses (Figure 1.11). The L-DNA segment of HHV-8 has many open reading frames with significant homology to human genes. The genome is circular during latent infection and linear during the lytic phase. Like other herpesviruses, replication is by the rolling circle mechanism within the nucleus of the host cells (Hengge *et al.*, 2002b).

Human herpesvirus-8 has been phylogenetically classified into strains A, B, C, D and E, and further divided into 13 subgroups based on amino acid differences (Biggar *et al.*, 2000). Subtypes A and C are very similar, while subtype B is the most divergent subtype. The A and C subtypes have a wide distribution throughout Europe, US, Asia and the Middle East. American patients with acquired immunodeficiency syndrome (AIDS) are usually infected with subgroup A1, A4 and C3, while subgroup A5 has been found in some African samples (Zong *et al.*, 1999). The B subtype is found predominantly in sub-Saharan Africa or in persons of African heritage and has only 3 subgroups (B1, B2 and B3) (Zong *et al.*, 2002). The D subtype appears to be rare and isolated to the Pacific Island region (Poole *et al.*, 1999). Sequences recovered from a Brazilian Amerindian population demonstrated a new type named type E (Biggar *et al.*, 2000). On the amino-acid level, HHV-8 has 30-50% homology to Epstein–Barr virus and herpesvirus saimiri sequences for 30-50% (Hengge *et al.*, 2002b).

1.22.2 Human herpesvirus-8 and malignancies

Human herpesvirus-8 (HHV-8) has been aetiologically linked to Kaposi's sarcoma (KS), primary effusion lymphomas (PELs) and multicentric Castleman's disease (MCD).

Kaposi's sarcoma is a vascular proliferation tumour that typically manifests as a mucocutaneous lesion. HHV-8 has been detected in spindle cells of KS tumours originating from endothelial cells. The four known types of KS are Classic KS (a generally benign cutaneous disease common among males resident around the Mediterranean), AIDS-associated KS (highly aggressive, attaking mucocutaneous surfaces and viscera), endemic KS (prevalent in non-HIV infected persons in some regions of Africa such as Uganda, and known to be more aggressive than the classic type of KS) and iatrogenic KS that has a variable clinical presentation related to the degree of therapy. It has been demonstrated that the prevalence of HHV-8 DNA among KS lesions is at least 95% (Cathomas, 2000; Cathomas, 2003; Wilkins *et al.*, 2006).

When HHV-8 infects the B-lymphocytes it causes two different types of malignancies, primary effusion lymphomas (PEL) and multicentric Castleman's disease (MCD).

Primary effusion lymphomas are malignant B cell lymphomas manifesting as body cavity effusions. The most significant criterion of the PEL is the positivity of HHV-8 DNA. It has been demonstrated that the prevalence of HHV-8 DNA reaches 100%, irrespective of the presence or absence of HIV disease (Nador *et al.*, 1996; Cathomas, 2000; Ablashi *et al.*, 2002)

Multicentric Castleman's disease is another B cell lymphoproliferative disease associated with HHV-8. Although MCD is considered as a prelymphoma state, it is not known whether there is a pathogenetic link between MCD and PEL. All examined AIDS–associated MCD lesions have detectable HHV-8, but only 50% of lesions of MCD in the absence of HIV contain HHV-8 (Ascoli *et al.*, 2001; Pagano *et al.*, 2004; Waterston and Bower, 2004).

In the last few years, oral plasmablastic lymphoma (PBL) has been suggested to be linked to HHV-8. Plasmablastic lymphoma is an aggressive neoplasm and has been listed in the WHO classification as a variant of diffuse large B cell lymphoma, although this classification has been denied recently by Vega *et al.*,2005. However, the link between HHV-8 and PBL was under investigation by many researchers (Delecluse *et al.*, 1997; Lin *et al.*, 2004; Folk *et al.*, 2006) suggesting that oral PBL in AIDS patients is strongly associated with HHV-8 and EBV (Cioc *et al.*, 2004). Furthermore, some studies have suggested that HHV-8 and EBV may have a possible pathological role in PBL in sites other than the oral cavity (Verma *et al.*, 2005) Of note, a case report of oral PBL in immunocompetent HIV-negative individuals did not detect any presence of HHV-8 or EBV (Lee *et al.*, 2006)

HHV-8 associated tumours initiation and progression is linked with cytokine-mediated reactivation of HHV-8, and may arise only in individuals with specific immunogenetic phenotype (Cathomas, 2003). HHV-8 tightly regulates expression of its genes; these genes can be divided into latent or lytic infection. HHV-8 genes expressed during latent infection can induce cell growth by activation of cell cycle genes and block apoptosis in virally infected

tumour cells. The functions of these virus proteins ensure life-long viral persistence in the host. Only small subset of tumour cells show evidence of viral lytic infection by expressing the whole range of putative oncogenes as detailed elsewhere (Schulz, 2000; Cathomas, 2003).

1.22.3 HHV-8 in salivary gland lesions

As with the previously mentioned herpesviruses, there are few data of the involvement of HHV-8 in the pathogenesis of salivary gland tumours. One early study did not detect HHV-8 in the tumour of pleomorphic-adenoma or malignant salivary gland tumours (Atula *et al.*, 1998). A more recent study did not find HHV-8 to be associated with benign lymphoepithelial cysts of the parotid gland (Yen *et al.*, 2004). Of note, however HHV-8 was detected in major salivary glands KS of HIV-disease although this of course is not unexpected (Castle and Thompson, 2000).

1.22.4 HHV-8 in oral lesions

Epidemiological and molecular studies have demonstrated the relationship of HHV-8 to Kaposi's sarcoma and plasmablastic lymphoma of the oral cavity (Castle and Thompson, 2000) in the mouth of HIV/KS patients (Chang *et al.*, 1994; Huang *et al.*, 1996; Hille *et al.*, 2002). Furthermore, HHV-8 has also been found in oral ulcer tissues of HIV-infected individuals who did not have KS (Di Alberti L. *et al.*, 1997). However, a recent study of Taiwanese patients did not find any association between HHV-8 and OSCC (Yang *et al.*, 2004).

1.23 Viruses in normal oral mucosa

The detection rates of HPV in normal oral mucosa in the literature range from 0% (Eike *et al.*, 1995; Cruz *et al.*, 1996; Bouda *et al.*, 2000; Sand *et al.*, 2000) to 70 % (Terai *et al.*, 1999). In one study up to 60% of normal volunteers had some form of HPV in their oral mucosa (43% with either type 16 or 18), although the detection rate varied depending upon whether buccal scrapings, biopsy tissues or mouthwash were examined (Lawton *et al.*, 1992). The number of the examined samples of healthy oral mucosa have sometimes been low or have comprised histopathologically normal tissue from patients with OSCC (Maitland *et al.*, 1987; Shindoh *et al.*, 1995). Furthermore, in some large surveys, normal epithelium has been collected by scraping normal mucosa and such samples may not include the basal layers which may contain latent HPVs (Sugiyama *et al.*, 2003).

Studies involving larger sample sizes than the aforementioned investigations have reported low (1-2%) prevalence rates of HPV-DNA in normal individuals (Lambropoulos *et al.*, 1997; Smith *et al.*, 1998), these studies have utilized PCR detection method, one of the most sensitive assays for HPV detection. Using similar techniques, investigators conducting a recent study in India of betel nut users found a detection rate of 33.6% among the normal control group (Nagpal *et al.*, 2002), raising the possibility that there are geographic, exposure-related or other behavioural influences that play a role in the acquisition of HPV within the normal oral mucosa (Ha and Califano, 2004).

Studies of herpesviruses such as EBV, CMV and HHV-8 have also yielded variable detection rates in normal oral mucosa. The presence of

HHV-8 has been investigated in different control groups including healthy blood donors (Bigoni *et al.*, 1996; Decker *et al.*, 1996; Blackbourn *et al.*, 1997). HHV-8 was assessed among healthy populations by seroprevalence of the virus, the highest being found in Sub-Saharan Africa whereby approximately 40% were seropositive for antibodies to HHV-8 (Ablashi *et al.*, 1999; Schulz, 2000). However, other studies did not detect HHV-8 in the saliva of healthy controls (Lucht *et al.*, 1998) or in the saliva of any HIV-negative patients (Boldogh *et al.*, 1996).

The detection of EBV-DNA was highly variable among normal healthy samples. EBV-DNA was not detected in samples from normal oral epithelium (Lin *et al.*, 2005), nor in the nasopharyngeal space of healthy control samples (Hao *et al.*, 2003), whilst it was found in 40% of healthy adults throat washing in Taiwan (Jeng *et al.*, 1994), and in 46.6% of the oral scrapes of a group of immunocompetent subjects resident in Brazil (Braz-Silva *et al.*, 2006).

Although cytomegalovirus seems to be rarely detected in normal healthy oral mucosa and healthy salivary glands (Ammatuna *et al.*, 2001; Laane *et al.*, 2002; Yang *et al.*, 2004), it has been present in the oral fluids of immunocompetent individuals (Beyari *et al.*, 2005).

In a recent study of the presence of EBV, CMV, HHV-8 and HPV in ulcerated oral tissues (Lin *et al.*, 2005), specimens of fresh normal oral mucosal tissues and sera were used as controls, no viral DNA being detected among the control samples. Another study has investigated the presence of EBV, CMV, HHV-8 and HPV among OSCC patients from Taiwan, no detection of any virus in the control group being reported (Yang *et al.*, 2004).

The cause of the differences in the detection rates of HPV and some herpesviruses remains to be clearly determined, nevertheless the majority of recent studies have used very careful and accurate techniques, and overall it seems that these studies suggest that HPV, CMV, EBV and HHV-8 are commonly present in healthy normal mucosa.

1.24 Viral interactions in OSCC

Interactions between viruses have the potential to decrease or more likely, enhance the potential pathogenic effects of individual viruses (Mbopi-Keou *et al.*, 2002). In particular, synergism between viruses may have the potential to increase any liability to clinical disease. For example, in HIV disease, HIV driven cell-mediated immunosuppression will increase the likelihood of other viral infections, particularly herpes group infections of the mouth (e.g. EBV and HHV-8). However, HIV infection may itself be enhanced by CMV and EBV and reduced by HHV-8, while HHV-6 and HHV-7 possess mechanisms that could either enhance or reduce HIV infection (Mbopi-Keou *et al.*, 2002). Recent studies using human epithelial culture tissues (organotypic graft) reveal that HSV (1 or 2) increased the copy number of HPV and that herpetic infection did not affect episomal status of HPV genomic DNA. Furthermore, HSV infection maintains expression of HPV E6 and E7 (essential for oncogenesis) but suppresses E1 and E2 (essential for HPV-DNA replication) (Meyers *et al.*, 2003). While these results do not suggest that HSV is a co-factor for HPV oncogenesis, they do indicate that there is the potential for a dynamic relationship between herpesviruses and HPV.

With respect to the oral mucosa, the demonstration of co-infection with CMV, HHV-8 in HPV infected individuals might suggest a synergistic oncogenic role of these viruses (Tsuhako *et al.*, 2000). Epstein-Barr virus is known to be able to encode a specific protein which suppresses cellular immune-responses, thus the simultaneous infection of epithelial cells by EBV and HPV (combined with reduced immune status) could increase the risk of

the development of potentially-malignant and malignant oral mucosa diseases compared with single viral infection (Rassekh *et al.*, 1998). Indeed infection with EBV, CMV and HPV has been suggested to play some role in the pathogenesis of some malignancies such as genital tract malignancy in females (Gradilone *et al.*, 1996), and bladder malignancy (Gazzaniga *et al.*, 1998).

1.25 Summary of herpesviruses and OSCC

Human herpesviruses have not yet been consistently detected in OSCC, although may be associated with a number of other head and neck malignancies. Hence, the evidence to suggest that such herpesviruses are associated with OSCC is limited and a causal association has not been demonstrated. Nevertheless, these viruses might have a role as a minor co-factor in the pathogenesis of OSCC.

1.26 Aims

Molecular and epidemiological data indicate that HPV may be a causal factor in a subset of oral, oropharyngeal and laryngeal carcinomas, and may cause malignant disease via the same mechanisms of oncogenesis as those of cervical malignancy (Lakshmi *et al.*, 1993; Gillison *et al.*, 1999; Gillison, 2004; Syrjanen, 2005; Szentirmay *et al.*, 2005; Kreimer *et al.*, 2005).

Associations of herpesviruses and oral potentially malignant and malignant tumours have been previously proposed. but there are little consistent data to suggest that these viruses commonly residing within the mouth may give rise to oral tumours (Yang *et al.*, 2004).

The aim of the present study was to investigate the DNA prevalence of HPV, CMV, EBV and HHV-8 in potentially malignant and malignant oral lesions of patients with a range of relevant diseases. In addition, the prevalence of these viruses was examined in salivary gland malignant diseases to establish if they may play any role in the development or perpetuation of such disease. Samples from two geographic locales were examined to establish whether there is any consistency in any observed associations.

Chapter 2

Patients, Materials and Methods

2.1 Patients

Eighty two archival, paraffin embedded tissues (PET), histopathologically confirmed oral specimens consisting of oral squamous cell carcinoma (30 samples), oral lichen planus (32 samples), mucoepidermoid carcinoma (10 samples), adenoidcystic carcinoma (4 samples) and pleomorphic adenoma (6 samples), were obtained from College of Dentistry, King Saud University and Riyadh Central Hospital (Kingdom of Saudi Arabia).

Another 196 archival samples were obtained from the pathology archives of the Eastman Dental Institute (EDI) UK consisting of oral epithelial dysplasia (45 samples), proliferative verrucous leukoplakia (12 samples), erosive/non-erosive oral lichen planus (30), oral squamous cell carcinoma (34), human immunodeficiency virus positive samples (25) and 50 samples of histologically diagnosed normal oral mucosa (Table 2.1).

The biopsy samples had been taken from different sites in the head and neck area including tongue (all aspects), hard and soft palate, upper and lower lip, submandibular region, retro-molar area, buccal mucosa, floor of the mouth, salivary glands, oropharynx, pre-maxilla and interdental papilla area.

The characteristics of the different tissue samples are summarized in tables 2.2 and 2.3.

Table 2.1 Summary of the lesional tissues and patients sources

Country	Lesion (No)	Gender (No.)	Median age (year)	Age range (year)	Ethnicity
UK					
	PVL (12)				
		F (6)	69	48:80	C= 4, N/A=2
		M (6)	72	57:85	C=1, N/A=5
	OLP (30)				
		F (14)	58	42:79	C=8, SEA=3, N/A=3
		M (16)	60	40:79	C=8, SEA=2, N/A=6
	OED (45)				
		F (22)	62	35:77	C=14, SEA=4, N/A=4
		M (23)	56	29:72	C=16, SEA=2, N/A= 5
	OSCC (34)				
		F (12)	70	56:90	C=6, SEA=6
		M (22)	68	31:96	C=20, SEA=1, N/A=1
	HIV (25)				
		F (3)	40	35:50	C=2, SEA=1
		M (22)	42	31:53	C=20, AF=1, N/A=1
Total	= 146	(F = 57, M = 89)	58.5	29:96	C=99, SEA=19, N/A=27, AF=1
KSA					
	OLP (32)				
		F (19)	43	23:65	AR=19
		M (13)	47	28:60	AR=10, SEA=1, FEA=2
	OSCC (30)				
		F (7)	66	45:80	AR=7
		M (23)	59	30:81	AR=21, C=2
	Salivary (20)				
		F (7)	42	25:61	AR=7
		M (13)	42	20:65	AR=11, SEA=1, FEA=1
Total	= 82	(F = 33, M = 49)	50	20:81	AR= 75, SEA=2, FEA=3, C=2
Normal Controls					
		F (29)	37	14:84	C= 23, SEA=5, AF=1
		M (21)	44	18:105	C=14, SEA=5, AR=2
Total	= 50	(F= 29, M= 21)	39.5	14:105	C=37, SEA=10, AF=1, AR=1

C=Caucasian, SEA=South East Asia, FEA=Far East Asia, AR=Arab, AF=African,
N/A= Data Not available, PVL= Proliferative verrucous leukoplakias, OLP= Oral lichen planus
OED=Oral epithelial dysplasia, OSCC= Oral squamous cell carcinoma,
HIV= Human immunodeficiency virus

Table 2.2 Characteristics of patients from the United Kingdom

Characteristics	OSCC		OED		PVL		OLP		HIV	
	F (%)	M (%)	F(%)	M (%)	F(%)	M(%)	F(%)	M(%)	F(%)	M(%)
Biopsy sites										
Buccal mucosa	4 (33)	2 (9)	3 (14)	5 (22)	4 (66)		11 (79)	14 (88)		4 (18)
Lat. of tongue *		5 (23)	6 (27)	6 (26)		2 (33)	2 (14)	1 (6)		2 (9)
Attached gingiva			1 (4.5)				1 (7)	1 (6)		2 (9)
Hard palate		1 (5)		2 (9)	1 (17)				2 (67)	3 (13.5)
Soft palate		1 (5)		2 (9)						
Lip	2 (17)	3 (14)	1 (4.5)	1 (4)	1 (17)				1 (33)	5 (23)
Commisure	1 (8)		3 (14)	6 (26)						3 (13.5)
Floor of the mouth		5 (23)	2 (9)	1 (4)		2 (33)				
Ant 2/3 of Tongue**	2 (17)		4 (18)			1 (17)				1 (5)
Retromolar		1 (5)	1 (4.5)			1 (17)				
Salivary			1 (4.5)							
Other sites***	3 (25)	4 (18)								2 (9)
Cigarette										
None	12 (100)	19 (86)	15(68)	9(39)	6(100)	4(67)	13(93)	13(81)	3(100)	15(68)
Ex-smoker				3 (13)				1 (6)		
Current smoker		3 (14)	7 (32)	11 (48)		2 (33)	1 (7)	2 (13)		7 (32)
Smoking/day										
None	12 (100)	19 (86)	15(68)	9(39)	6(100)	4(67)	13(93)	13(81)	3(100)	15(68)
<5							1 (7)			
10-15/day		2 (9)	4 (18)	8 (35)				2 (13)		
>20/day		1 (5)	3 (14)	6 (26)		2 (33)		1 (6)		7 (32)
Paan										
None	10 (83)	20 (91)	19(86)	21(91)	6(100)	5(83)	13(93)	16(100)	3(100)	22(100)
Current user	2 (17)	2 (9)	3 (14)	2 (9)		1 (17)	1 (7)			
Alcohol										
None	12 (100)	18 (81)	11(50)	12(52)	6(100)	5(83)	12(86)	11(69)	3(100)	21(96)
<5 u/w!!			3 (14)	1 (4)						
5-20 u/w		1 (5)	1 (4.5)	2 (9)			1 (7)	1 (6)		
>20u/w			6 (27)	6 (26)		1 (17)		2 (12.5)		1 (4)
N/A***		3 (14)	1(4.5)	2(9)			1(7)	2(12.5)		
Corticosteroids										
None	12 (100)	22(100)	22(100)	23(100)	6(100)	6(100)	11(67)	15(94)	2(67)	16(73)
Topical							1 (7)			
Systemic							2 (14)	1 (6)	1 (33)	6 (27)
Immunologically related diseases										
None	10 (83)	22(100)	21(95)	22(96)	6(100)	6(100)	9(65)	12(75)		1(4)
Systemic disease!	2 (17)		1 (5)	1 (4)			2 (14)	2 (12.5)	3(100)	21(96)
Immunosuppressive drugs							3 (21)	2 (12.5)		
Relevant medical history										
None	4 (33)	11(50)	11(50)	9(39)	2(33)	1(17)	5(37)	12(75)	3(100)	17(77)
HRT¶¶			5 (23)				2 (14)			
Asthma				2 (9)			2 (14)			
Candida		1 (5)	2 (9)	3 (13)		1 (17)	1 (7)	2 (12.5)		4 (18)
Cutaneous disease							2 (14)	2 (12.5)		1 (5)
Diabetes mellitus				1 (4)			2 (14)			
OSCC¶	8 (67)	10(45)	2 (9)	5 (22)	3 (50)	3 (50)				
Penicillin allergy					1 (17)	1 (17)				
Submucous fibrosis			2 (9)	2 (9)						
Arthritis				1 (4)						
Therapies										
None	8 (67)	18 (81)	18(82)	23(100)	5(83)	6(100)	14(100)	16(100)	3(100)	16(100)
Chemotherapy	3 (25)	3 (14)	4 (18)		1 (17)					
Radiotherapy	1 (8)	1(5)								
Triple therapy										6 (27)
Total	**12**	**22**	**22**	**23**	**6**	**6**	**14**	**16**	**3**	**22**

* Lt. of tongue= lateral border of the tongue, ** Ant. 2/3 of tongue= anterior 2/3 of tongue
*** Other sites= Data not available, ¶OSCC= Second-primary Oral Squamous Cell Carcinoma, ¶¶ HRT= Hormone replacement therapy
!Systemic immune disease=e.g. Stevens Johnson's syndrome, Benign mucous membrane pemphigoid.!!u/w= units per week

Table 2.3 Characteristics of patients from the Kingdom of Saudi Arabia

Characteristics	OSCC		OLP		Salivary diseases	
	F(%)	M(%)	F(%)	M(%0	F(%)	M(%)
Biopsy sites						
Buccal mucosa	2(29)	4(18)	17(90)	10(76)	1(14)	4(30)
Lt. of tongue*	1(14)	6(26)		1(8)		
Attached gingiva			1(5)			
Hard palate					3(44)	6(46)
Soft palate						
Lip		1(4)		1(8)	1(14)	
Floor of the mouth		2(9)				1(8)
Ant 2/3 of Tongue	1(14)			1(8)		
Retromolar	1(14)	7(30)			1(14)	1(18)
Other sites**	2(29)	3(13)	1(5)		1(14)	1(18)
Cigarette						
None	7(100)	22(96)	19 (100)	13 (100)	7(100)	13(100)
Current smoker		1(4)				
Smoking/day						
None	7(100)	22(96)	18 (95)	13 (100)	7(100)	13(100)
10-15/day		1(4)	1 (5)			
Paan						
None	7(100)	23(100)	19 (100)	13 (100)	7(100)	13(100)
Alcohol						
None	7(100)	23(100)	19 (100)	13 (100)	7(100)	13(100)
Corticosteroids						
None	7(100)	23(100)	19 (100)	13 (100)	7(100)	13(100)
Immunologically related disease						
None	7(100)	23(100)	19 (100)	13 (100)	7(100)	13(100)
Relevant medical history						
None	5(72)	20(88)	12 (63)	8 (62)	7(100)	13(100)
Asthma			1(5)			
Cutaneous Disease			3(16)	3(23)		
Diabetes mellitus	1(14)	1(4)	3(16)	2(15)		
Second/primary OSCC	1(14)	1(4)				
Penicillin allergy		1(4)				
Therapies						
None	7(100)	23(100)	19 (100)	13 (100)	7(100)	13(100)
Total	**7**	**23**	**19**	**13**	**7**	**13**

* Lt of tongue= lateral border of tongue
**other site= No recorded data

2.2 Materials

2.2.1 Laboratory accommodation

PCR–related procedures were performed in four separate laboratory areas in the Health Protection Agency, Colindale, London-UK:

- A clean room used for preparing reagents (except DNA)
- An amplification room used to run the PCRs
- A gel room for the detection of PCR amplicons by gel electrophoresis

The workflow was strictly unidirectional in order to prevent contamination with PCR products. Samples and plasmids containing high copy numbers of HPV DNA were handled in a separate laboratory remote from the designated areas.

2.3 Methods

2.3.1 DNA Extractions

Paraffin embedded tissue (PET) were sectioned using a sledge microtome (AS300, Shandon, UK), serial sections of ≈ 5–8 μm thickness, folded and inserted in 1.5 ml eppendroff tubes (Sarstedt, Germany).

Samples were sent to Genovar Company. DNA was extracted using a commercially available kit "Genovar Nucleic Acid Extraction Kit for Paraffin Embedded Tissue" Genovar, UK. The kit is designed to extract total nucleic acid from formalin-fixed, paraffin embedded tissue, and is optimised for

extraction of DNA from one section of the tissue up to 20 µm thickness. (Appendix 2.1)

2.3.2 PCR amplification of β–globin DNA

The presence of DNA in extracts from PET was verified by the amplification and detection of a 268 bp fragment of the housekeeping β-globin gene as an indicator for the successful extraction. The PCR was carried out in a 25 µl reaction mixture containing 2.5 µl PCR buffer (Invitrogen, Paisley U.K, 10X buffer II), 0.75 µl of 50 mM $MgCl_2$, 1 µl dNTP's (Invitrogen, Paisley U.K), 1 µl (20 pmol) of each β-globin primers "PC04 and GH20" (Cruachem Ltd, Glasgow UK) was added, 0.2 µl of *Taq* DNA polymerase (5U/µl) (Life technologies), and16.55 µl of water. Twenty three µl of the mix was added with 2 µl of DNA extract. Single round PCR was then applied, thermal cycler (Biometra T3 Thermocycler, Germany) being programmed to 94ºC for 5 m, followed by 94ºC for 1 m (denaturation), 55ºC for 1 m (annealing), and 72ºC for 1 m (extension), and the cycle being repeated for 34 times (Gravitt *et al.*, 1998; Fredricks and Relman, 1999; Sato *et al.*, 2001).

2.3.2.1 Oligonucleotide primers for the amplification of β-globin DNA

PC04: 5`- CAACTTCATCCACGTTCACC-3`
GH20:5`- GAAGAGCCAAGGACAGGTAC-3`

The length of the β–globin product ≈ 268 bp (Gravitt *et al.*, 1998; Fredricks and Relman, 1999; Sato *et al.*, 2001)

2.3.3 PCR detection of HPV L1 DNA

The viral genome encompasses three main segments of unequal sizes. About 10% of the HPV genome corresponds to the long control region (LCR), and its function is the regulation of viral gene expression. The other part of the genome contains the early (E) or the late (L) genes. The L1 and L2 genes encode the major and minor capsid proteins, which are only expressed in terminally differentiated squamous epithelial cells. The DNA base sequence of L1 gene diverges only slightly in different HPV species, giving the molecular basis to detect different virus species by single polymerase chain reaction. The L1 consensus primers detect the presence of episomal forms of the HPV, predominantly present in oral cancers (Maitland *et al.*, 1987). The integrated form of the virus is generally associated with cervical cancers, and often results in disruption of the L1 gene (de Villiers *et al.*, 1985; Maitland *et al.*, 1987; Jeon *et al.*, 1995). In addition, L1 gene regulates levels of HPV E6 and E7 gene expression, which are important in tumourogenesis, due to interaction and inactivation of the growth suppressor genes p53 and Rbp proteins (D'Costa *et al.*, 1998).

2.3.3.1 Oligonucleotide primers of HPV

The high degree of sequence diversity between HPV types prevents the selection of common oligonucleotide primers for the simultaneous detection of all HPV types by PCR. A PCR with the "degenerate" consensus primers MY09/MY11 (Cruachem Ltd, Glasgow UK) initially described by Manos *et al* (1998), for the detection of genital HPVs, is also widely used to analyse skin lesions (McGregor *et al.*, 1994). The "general" primers

GP5$^+$/GP6$^+$ (Cruachem Ltd, Glasgow UK), (Roda Husman *et al.*, 1995) have proved to be successful for the detection of a broad spectrum of HPV genotypes in cervical samples (Qu *et al.*, 1997). A significant increase in sensitivity of detection was observed when all four primers (MY09/MY11, GP5$^+$/GP6$^+$) were used in a nested PCR (Evander *et al.*, 1992; Strauss *et al.*, 2000).

All optimisations of PCRs were performed using CaSki cells which contain 600 copies of HPV type 16 DNA per cell ((cell lines were obtained from the European Collection of Cell Culture, (ECACC, cat No.87020501)). A limited number of experiments were performed using HeLa cells which contain 1 copy of HPV type 18 DNA per cell (ECACC, cat No. 93021013) (Strauss *et al.*, 2000).

2.3.3.2 PCR amplification of HPV (ORF-L1) DNA

A nested protocol was performed to amplify subgenomic HPV (ORF-L1) DNA. First round PCR was carried out using degenerate primers MY09 and MY11 for the initial amplification (with an expected product size of about 450 bp). Fifty µl of reaction mix was prepared, each contains 4 µl of PCR buffer (Invitrogen, Paisley U.K), 1 µl of 50 mM $MgCl_2$, 0.4 µl of dNTP's (Invitrogen, Paisley U.K). Then L1 primers (20 pmol each) 1 µl of MY 09 and 1 µl of MY 11 were added, followed by 0.25 µl of *Taq* DNA polymerase (5U/µl) (Life Technologies) and 32.35 µl of water. Forty µl of the mix was added to 10 µl of the DNA extract, covered with a drop of mineral oil and then subjected to the thermal cycler. The mixtures were incubated for 5 m at 94°C for DNA denaturation followed by 40 cycles of amplification. Each cycle

consisted of a denaturation step at 94℃ for 1 m, an annealing step at 55 ℃ for 2 m and an elongation step at 72 ℃ for 3 m. The last cycle was extended 7 m for elongation at 72 ℃. The second round of amplification with the general primers $GP5^+$ and $GP6^+$ (Roda Husman *et al.*, 1995) positioned inside of the MY09 and MY11 primers was performed on aliquot of the first round product; these amplified a 150 bp products. PCR was carried out in 50 µl reactions each containing 4 µl of PCR buffer, 7 µl of 50 mM $MgCl_2$, 0.7 µl of dNTP's, and then add L1 primers (20 pmol each) 1 µl of $GP5^+$ and 1 µl of $GP6^+$ were added, followed by 0.2 µl of *Taq* DNA polymerase (5U/µl), and 35.1 µl of water. Forty-eight µl of the mix was added to 2 µl of the previous PCR product, covered with one drop of mineral oil and subjected to the thermal cycler. Forty cycles of amplification were carried out; each cycle consists of a 1 m denaturation step at 94℃ and annealing step at 40℃ for 2 m and an elongation step at 72 ℃ for 1.5 m. The first cycle was preceded by 4 m of denaturation at 94℃ and the last cycle was extended by 4 m elongation at 72℃.

2.3.3.3 Oligonucleotide primers for the amplification of HPV-L1

MY 09: 5`-CGTCCMARRGGAWACTGATC-3`
MY 11: 5`-GCMCAGGGWCATAAYAATGG-3`
GP 5+: 5`- TTTGTTACTGTGGTAGATACTAC-3`
GP 6+: 5`- GAAAAATAAACTGTAAATCATATTC-3`

The length of the HPV DNA product was 450 bp at the 1st round and 150 bp at the nested round.

2.3.4 PCR amplification of the HHV-8 ORF-K1

First round PCR amplification of the first variable region of KSHV ORF-K1 was carried out in a 50 µl reaction mixture containing 33.8 µl of nuclease free water, 5µl buffer number 8 (Stratagene Europe, The Netherlands) containing 3.2 mM $MgCl_2$, 1µl of each outer primer, 1 µl of each dNTP's and 0.2 µl of *Taq* DNA polymerase. To this mixture 5 µl of extracted DNA was added. The second round conditions were the same as the first with the addition of 1µl of each inner primer and 2 µl of the primary product as template. Amplification of both regions was carried out under the same PCR thermocycling conditions. Samples were heated to 94℃ for 5 m followed by 35 cycles of 94℃ for 1 m, 60° for 1 m and 72℃ for 1 m followed by 5 m of extension period at 72℃ (Raab *et al.*, 1998; Cook *et al.*, 2002a)

2.3.4.1 Oligonucleotide primers for the amplification of KSHV -K1

K1 inn 5: 5`- CCCTGGAGTGATTTCAACGC-3`
K1 inn 6: 5`- ACATGCTGACCACAAGTGAC-3`
K1-1: 5`- GAGTGATTTCAACGCCTTAC-3`
K1-N: 5`- TGCTGACCACAAGTGACTGT-3`

The ORF-K1 outer primers produced a 255 bp fragment, and the inner primers produced a 247 bp fragment. (Cook *et al.*, 2002a).

2.3.5 PCR amplification of the CMV-gN DNA

A nested protocol was performed to amplify subgenomic CMV-gN region DNA. First round PCR was carried out in 50 µl reactions each containing, 5 µl of PCR buffer, 8 µl of 50 mM $MgCl_2$, 4 µl of dNTP's, then first round gN primers (20 pmol each) 2 µl of gN-up, 2 µl of gN-low were added,

followed by 0.38 µl of *Taq* DNA polymerase (5U/µl), and 23.62 µl of water. Forty-five µl of the mix was added to 5 µl of the DNA extract covered with a drop of mineral oil, the nested round is identical to the first one except in 26.62 µl of water was added followed by second round gN primers(20 pmol each), 2 µl of gN-3 and 2 µl gN-4. Forty-eight µl of the mix was added to 2 µl of the first round as template, and then subjected to the second round PCR. The thermal cycler (both rounds have the same protocols) at 96ºC for 1 m, then at 96ºC for 1 m, then at 55ºC for 1 m, then at 72ºC for 1 m, then the cycle was repeated for 35 times, finally at 72ºC for 10 m (Pignatelli *et al.*, 2001; Pignatelli *et al.*, 2003).

2.3.5.1 Oligonucleotide primers for the amplification of CMV-gN

gN-up: 5'- TGGTGTGATGGAGTGGAAC-3'
gN-lw: 5'- TAGCCTTTGGTGGTGGTTGC-3'
gN-3: 5'- GTAGTGGCAGAGAGTTCTGG-3'
gN-4: 5'- GTACAATACAAAAAGCTCCC-3'

The second round product was 304 bp in length.

2.3.6 PCR amplification of the (EBV-*Bam*HI K) DNA

Primers were selected to allow amplification of *Bam*HI K region. PCR was performed on 50 µl reaction mixture, first round consisted of 5µl of PCR buffer, 2µl of each primer at 20 pmol, 1.5 µl of 50 mM $MgCl_2$, 0.25 of *Taq* DNA polymerase, 1µl of dNTP's and 33.25µl of water, topped with 5µl of DNA template. PCR was performed for 35 cycles (denaturation at 94 ºC for 1 m, annealing at 68 ºC for 40 s and extension at 72 ºC for 1 m). Before the start of each reaction, the samples were denatured for 5 m at 94 ºC; after the last cycle, the extension step was extended by 10 m. Further amplification

was carried out by adding 2 µl of the first round to 48 µl of second round PCR mix, which contained the inner primers of the *Bam*HI K amplicons. PCR was carried out for 1 m at 94 ℃, 40 s at 60 ℃, 1 m at 72 ℃ for 25 cycles (Triantos *et al.*, 1998).

2.3.6.1 Oligonucleotide primers for the amplification of EBV BAMHI-K

Outer, sense	TGATGGAGGCAGGCGCAAAAAAG
Outer, anti-sense	GAAACCAGGGAGGCAAATCTACT
Inner, sense	CGCAAAAAAGGAGGGTGGTTT
Inner, anti-sense	CATCGTCAAAGCTGCACACAG

The first round product was 469 bp, second round product was 433 bp in length.

2.3.7 Detection of PCR product

Eight µl of the PCR product was mixed with 2 µl of Orange G loading dye and loaded onto 2% agarose gel (SB fine gel, Severn Biotech Ltd.) along with 1 µg of 1 kb DNA ladder marker (Invitrogen, Paisley U.K) on one side of the gel. A positive and a negative controls were also been added. Electrophoresis took place in a 1X tris-borate EDTA buffer (TBE supplied as 10X stock) (Invitrogen, Paisley U.K). The gels were then stained with 5µg/ml ethidium bromide solution (Sigma, UK) in TBE buffer. DNA fragments were visualised using a short wave ultra-violet trans-illuminator and photographed using a photo printer.

2.3.8 *Reverse Line Blotting for HPV typing*

The method was based on the use of HPV-specific primers containing a hairpin loop structure in which fluorescent donor and quencher groups are

held in close proximity such that fluorescence is quenched. Amplification of the target sequence results in the opening of the loop. Fluorescent amplicons were typed by a single hybridization with specific probes immobilised in lines on a nylon membrane and detected by fluorescent scanner (Jordens *et al.*, 2000)

2.3.8.1 Molecular beacon primer-based PCR amplification and detection

Molecular beacon labelled primers was first used by Nazarenko *et al.*, 1997, and has been modified by developing a PCR method which permits the real-time detection of any HPV Infection (Jordens *et al.*, 2000), the fluorescent amplicons detected in positive samples were suitably labelled for direct testing against a panel of HPV genotype-specific probes. PCR using second round primers ($GP5^+$, $GP6^+$) for all the HPV positive samples was performed. The $GP6^+$ primer sequence was:

$GP6^+$: 5' F-GAAAAATAAACTGTAAATCATATTC 3'

F= Fluorescein.

The same conditions and protocols for the second round PCR in thermal cycler were used. The hairpin loop of a DNA sequence was added to the 5' end of the antisense $GP6^+$ primer sequence to give maximum fluorescence above background when incorporated into an amplicon (Nazarenko *et al.*, 1997). The primer sequence that contained a 5' fluorescein residue (F) and a quencher (Q) was synthesized by Oswel DNA services (Eurogenetc.UK). (Figure 2.1)

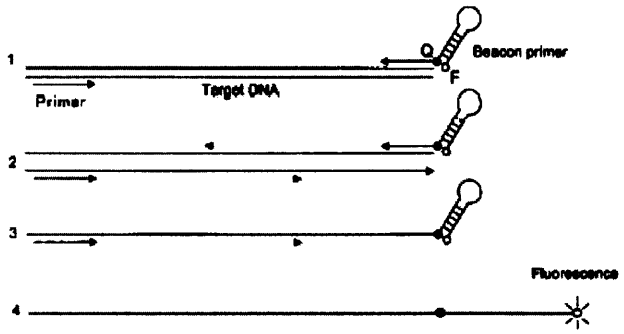


Figure 2.1 Showing the incorporation and opening out of molecular beacon primers during amplification, leading to fluorescence. (1), annealing of unlabelled and molecular beacon primers; (2), annealing and extension from primers; (3), annealing of unlabelled primer to molecular beacon primed DNA with extension leading to fluorescence (4) Q, quencher; F, fluorescein.
(Adapted from Jordens *et al.*, 2000)

2.3.8.2 Probe design and attachment to nylon membranes

Type-specific probes for 25 common HPV types were designed within the $GP5^+/GP6^+$ amplification regions of the L1 gene (sense) (Jordens *et al.*, 2000). The sequences (and references) of the probes are shown in Table 2.4. Probes were attached to the Biodyne nylon membranes (Pall BioSupport, UK) immediately before use. The denatured products were added to the prepared membrane, which has been activated in 16% EDAC (Sigma, UK) for 15 m, rinsed in water and placed in a mini-blotter (Biometra Ltd, UK). Optimum coupling of probes to the membrane was achieved by adding 14 µl of thawed amino-linked oligonucleotide probes to 346 µl fresh probe buffer (500 mM $NaHCO_3$= 0.84g $NaHc0_3$ + 20 ml DW), add 180 of diluted probe to the bottom slots of the mini-blotter for 1 m only, and then aspirated from the top slots, until all the probes finished. The membrane was then removed from the blotter, inactivated in 100 ml of 100 mM NaOH for 10 m, rinsed in water and then incubated in preheated sample buffer, 2× SSPE;

0.1% SDS (200 ml of sample buffer = 198 ml 2XSSPE + 2 ml 10% SDS) for 10 m at 56℃. After a final rinse in 2× SSPE membranes were used immediately or stored at 4℃ for subsequent use (Jordens *et al.*, 2000).

Table 2.4 Probe sequences for reverse line blotting

HPV type	Probe sequences (5`-3`)	References
6	ATCCGTAACTACATCTTCCACATACACCAA	Jacobs *et al.*,1995
11	ATCTGTGTCTAAATCTGCTACATACACTAA	Jacobs *et al.*,1995
16	GTCATTATGTGCTGCCATATCTACTTCAGA	Jacobs *et al.*,1995
18	TGCTTCTACACAGTCTCCTGTACCTGGGCA	Jacobs *et al.*,1995
31	TGTTTGTGCTGCAATTGCAAACAGTGATAC	Jacobs *et al.*,1995
33	TTTATGCACACAAGTAACTAGTGACAGTAC	Jacobs *et al.*,1995
35	GTCTGTGTGTTCTGCTGTGTCTTCTAGTGA	Jacobs *et al.*,1995
39	TCTACCTCTATAGAGTCTTCCATACCTTCT	Jacobs *et al.*,1995
42	CTGCAACATCTGGTGATACATATACAGCTG	Jacobs *et al.*,1995
43	TCTACTGACCCTACTGTGCCCAGTACATAT	Jacobs *et al.*,1995
45	ACACAAAATCCTGTGCCAAGTACATATGAC	Jacobs *et al.*,1995
51	AGCACTGCCACTGCTGCGGTTTCCCCAACA	Jacobs *et al.*,1995
52	TGCTGAGGTTAAAAAGGAAAGCACATATAA	Jacobs *et al.*,1995
53	CCGCAACCACACAGTCTATGTCTACATATA	Jordens *et al.*,2000
54	TACAGCATCCACGCAGGATAGCTTTAATAA	Jordens *et al.*,2000
56	GTACTGCTACAGAACAGTTAAGTAAATATG	Jordens *et al.*,2000
58	ATTATGCACTGAAGTAACTAAGGAAGGTAC	Jordens *et al.*,2000
59	GTGCTTCTACTACTTCTTCTATTCCTAATGTAT	Jordens *et al.*,2000
62	CTGCTGCAGCAGAATACACGGC	Jordens *et al.*,2000
66	ATTAATGCAGCTAAAAGCACATT	Hildeshein *et al.*, 1994
67	CTGAGGGAAAATCAGAGGCTAC	Qu *et al.*, 1997
70	CTGCACCGAAACGGCCATAC	Jordens *et al.*,2000
72	GCCACAGCGTCCTCTGTATCAGA	Jordens *et al.*,2000
han831	GTGCCACACAAACACCCTCTGA	Jordens *et al.*,2000
CP8304	CACAGCTACATCTGCTGCTGCAGA	Jordens *et al.*,2000

(Adapted from Jordens *et.al.*, 2000)

2.3.8.3 Hybridisation and detection by reverse line blotting

Twelve µl of amplification products were added immediately before use to 168 µl of preheated sample buffer (2× SSPE;0.1% SDS), denatured by boiling for 10 m in 100℃ heating block, then placed immediately on ice for 1 m. After a brief spin, samples were brought to room temperature. The prepared membrane was placed in the mini-blotter, turned through 90° relative to its original position so that each slot crossed all the probes. Then 180 µl of the denatured products were added to the slots, the mini-blotter placed in a plastic bag with damp tissue and incubated for 1 to 1.5 h at 60℃

to allow hybridisation. Samples were then removed by aspiration. The membrane removed from the blotter and washed twice with 150 ml wash buffer, 2× SSPE; 0.5% SDS (for 500 ml= 475 ml 2x SSPE+ 25 ml 10% SDS). Then rinsed in wash buffer B, 100 mM Tris-HCL, 150 Mm NaCl pH 7.5 (for 1 L= 12.1g Tris +8.77g NaCl) and incubated with Anti-fluorescein-peroxidase conjugate (Boehringer Ingelheim Limited, U.K) diluted in Liquid Blocking Reagent (1 in 2333) (Amersham Biosciences UK) and Wash buffer B (30 µl, 7 ml, and 63 ml respectively) for 30 m. The conjugate was then aspirated, followed by three washes with Wash Buffer B containing 0.3% Tween 20. Probe-target hybrids were detected by enzyme-linked chemifluorescence using the Opti-4CN (Bio RAD, UK) by mixing 2 ml of Opti-4CN Diluent, 18 ml DW and 400 µl of substrate. Then the liquid was poured into the mini-blotter with the membrane, left for a while until the dots appear and visually interpreted. The membrane was then photocopied by photo-printer (Figure 2.2). (Jordens *et al.*, 2000)

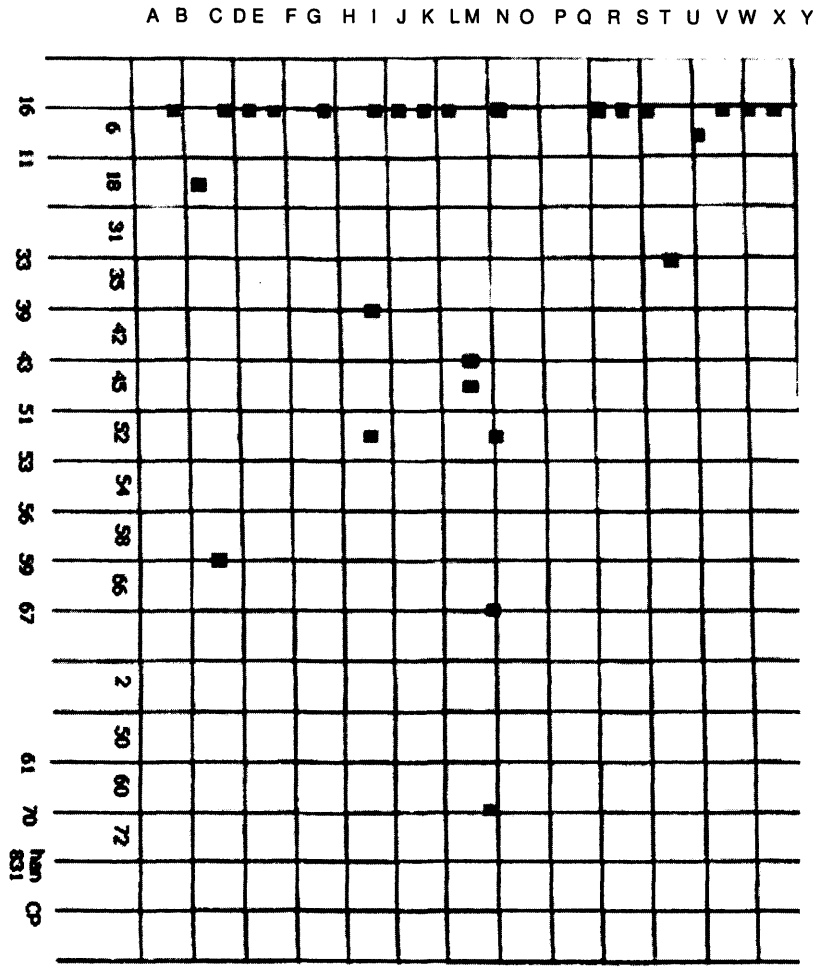


Figure 2.2 An example of a reverse line blot, showing single and mixed infections. The numbers indicate the probes (arranged vertically) and the letters represent the clinical samples (arranged horizontally). e.g., sample (I) has mixed infection of HPV types 16, 39 and 52

2.4 Statistical analysis

- Data entry and analysis were performed using the SPSS for Windows (Statistical Package of Social Science) software, version 12.0.

  Corporate Headquarters

  SPSS Inc.

  2335 Waker Drive, 11th Floor

  Chicago, IL

- Frequency distribution and cross-tabulation tables were constructed in order to analyze the demographic information, showing median age, mean age, age range and percentages of gender and ethnicity.
- A logistic regression model was used to determine the strength of any viral associations. Crude and adjusted (for risk factors) odds ratios (OR), and their 95% confidence intervals (CI) were calculated where possible. P-values have only been presented on those occasions where it was either not-possible or not-appropriate to present 95% CI for the parameter estimate, for example, where there was a zero in any of the cells of a 2x2 table.
- Bivariate correlation (Spearman's rank correlation coefficient) was used to test for viral interactions within OSCC.

Chapter 3

Human Papillomavirus- Results

Table 3.1 Frequency of detection of human papillomavirus in examined oral and salivary glands lesions

Country	Lesion	Gender	-ve No. (%)	+ve No. (%)
UK				
	OSCC	All	7/34 (20.6)	27/34 (79.4)
		F	2/12 (16.7)	10/12 (83.3)
		M	5/22 (22.7)	17/22 (77.3)
	PVL	All	5/12 (41.7)	7/12 (58.3)
		F	26 (33.3)	4/6 (66.7)
		M	3/6 (50.0)	3/6 (50.0)
	OED	All	26/45 (57.8)	19/45 (42.2)
		F	12/22 (54.5)	10/22 (45.5)
		M	14/23 (60.9)	9/23 (39.1)
	OLP	All	21/30 (70.0)	9/30 (30.0)
		F	10/14 (71.4)	4/14 (28.6)
		M	11/16 (68.8)	5/16 (31.3)
	HIV	All	16/25 (64.0)	9/25 (36.0)
		F	2/3 (66.7)	1/3 (33.3)
		M	14/22 (63.6)	8/22 (36.4)
KSA				
	OSCC	All	13/30 (43.3)	17/30 (56.7)
		F	2/7 (28.6)	5/7 (71.4)
		M	11/23 (47.8)	12/23 (52.2)
	OLP	All	23/32 (71.9)	9/32 (28.1)
		F	14/19 (73.7)	5/19 (26.3)
		M	9/13 (69.2)	4/13 (30.38)
	Salivary	All	15/25 (75.0)	5/25 (25.0)
		F	5/7 (71.4)	2/7 (28.6)
		M	10/13 (76.9)	3/13 (23.1)
Normal Controls				
	UK	All	50/50 (100.0)	0/50 (0.0)
		F	29/29 (100.0)	0/29 (0.0)
		M	21/21 (100.0)	0/21(0.0)

OSCC = Oral squamous cell carcinoma
PVL = Proliferative verrucous leukoplakia
OED = Oral epithelial dysplasia
OLP = Oral lichen planus
HIV = Human immunodeficiency virus

Table 3.2 Detection of human papillomavirus versus demographics and risk factors for oral squamous cell carcinoma of UK patients with oral epithelial dysplasia

Characteristic	-ve. NO. (%)	+ ve. No. (%)	–ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Gender						
Female	2/12(16.7)	10/12 (83.3)				
Male	5/22(22.7)	17/22 (77.2)				
Age (years)						
30-40	1/1(100)	0/1(0.0)		-	1/1 (100.0)	0/1(0.0)
50-60	2/8(25.0)	6/8(75.0)	0/4(0.0)	4/4(100.0)	2/4(50.0)	2/4(50.0)
60-70	1/5(20.0)	4/5(80.0)	0/2(0.0)	2/2(100.0)	1/3(33.3)	2/3(66.7)
70-80	1/12(8.3)	11/12(91.7)	1/2(50.0)	1/2(50.0)	0/10(0.0)	10/10(100.0)
>80	2/8(25.0)	6/8(75.0)	1/4(25.0)	3/4(75.0)	1/4(25.0)	3/4(75.0)
Ethnicity						
Caucasian	5/26(19.2)	21/26(80.8)	1/6(16.7)	5/6(83.3)	4/20(20.0)	16/20(80.0)
SEA*	1/7(14.2)	6/7 (85.7)	1/6(16.7)	5/6(83.3)	0/1(0.0)	1/1(100.0)
N/A	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Tobacco						
None	7/31(22.5)	24/31(77.4)	2/12(16.7)	10/12(83.3)	5/19(26.3)	14/19(73.7)
Current	0/3(0.0)	3/3(100.0)	-	-	0/3(0.0)	3/3(100.0)
Smoking						
None	7/31(22.5)	24/31(77.4)	2/12(16.7)	10/12(83.3)	5/19(26.3)	14/19(73.7)
10-15/day	0/2(0.0)	2/2(100.0)	-	-	0/2(0.0)	2/2(100.0)
>20/day	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Paan						
None	6/30(20.0)	24/30 (80.0)	2/10(20.0)	8/10(80.0)	4/20(20.0)	16/20(80.0)
Current	1/4(25.0)	3/4 (75.0)	0/2(0.0)	2/2(100.0)	1/2(50.0)	1/2 (50.0)
Alcohol						
None	6/30(20.0)	24/30(80.0)	2/12(16.7)	10/12(83.3)	4/18(22.2)	14/18(77.8)
5-20 u/w**	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
N/A***	1/3(33.3)	2/3(66.7)	-	-	1/3(33.3)	2/3(66.7)
Total	**7/34(20.6)**	**27/34(79.4)**	**2/12(16.7)**	**10/12(83.3)**	**5/22 (22.7)**	**17/22(77.3)**

*SEA=South East Asian
** u/w = units /week
*** N/A= Data not available

Table 3.3 Cross tabulation for human papillomavirus in UK-oral squamous cell carcinoma samples according to biopsy site, relevant medical note, corticosteroids, immune status and therapies

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Biopsy site						
Buccal mucosa	2/6(33.3)	4/6 (66.7)	2/4(50.0)	2/4 (50.0)	0/2(0.0)	2/2(100.0)
Lt. of tongue*	2/5(40.0)	3/5(60.0)	-	-	2/5(40.0)	3/5(60.0)
Floor of mouth	1/5(20.0)	4/5(80.0)	-	-	1/5(20.0)	4/5(80.0)
Soft palate	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Hard palate	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Ant. 2/3 of tongue**	0/2(0.0)	2/2(100.0)	0/2(0.0)	2/2(100.0)	-	-
Lip	0/5(0.0)	5/5(100.0)	0/2(0.0)	2/2(100.0)	0/3(0.0)	3/3(100.0)
Commisure	0/1(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	-	-
Retromolar	0/1(0.0)	1/1(100.0)	-		0/1(0.0)	1/1(100.0)
Other sites***	2/7(28.6)	5/7(71.4)	0/3(0.0)	3/3(100.0)	2/4(50.0)	2/4(50.0)
Relevant medical history						
None	2/15(13.3)	13/15(86.7)	0/4(0.0)	4/4(100.0)	2/11(18.2)	9/11(81.8)
SCC¶	5/18(27.8)	13/18(72.2)	2/8(25.0)	6/8(75.0)	3/10(30.0)	7/10(70.0)
Candidal infection	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Corticosteroids						
None	7/34(20.6)	27/34(79.4)	2/12(16.7)	10/12(83.3)	5/22(22.7)	17/22(77.3)
Immune status						
None	7/32(21.9)	25/32(78.1)	2/10(20.0)	8/10(80.0)	5/22(22.7)	17/22(77.3)
Systemic	0/2(0.0)	2/2(100.0)	0/2(0.0)	2/2(100.0)	-	-
Therapies						
None	4/26(15.4)	22/26(84.6)	1/8(12.5)	7/8(87.5)	3/18(16.7)	15/18(83.3)
Chemotherapy	2/6(33.3)	4/6(66.7)	1/3(33.3)	2/3(66.7)	2/3(66.7)	2/3(66.7)
Radiotherapy	1/2(50.0)	1/2(50.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	0/1(0.0)
Total	**7/34(20.6)**	**27/34(79.4)**	**2/12(16.7)**	**10/12(83.3)**	**5/22(22.7)**	**17/22(77.3)**

* Lt. of tongue= lateral border of tongue
** Ant. 2/3 of tongue= anterior 2/3 of tongue
*** Other sites= Data not available
¶SCC= Second-primary Squamous Cell Carcinoma

Table 3.4 Human papillomavirus infection in relation to UK-oral squamous cell carcinoma

UK	Gender				Odds Ratio (95 % CI)	P-value*
OSCC						
	ALL		Cancer			
			-	+		
		HPV -	50	7	N/C	<0.001
		HPV +	0	27		
	F		Cancer			
			-	+		
		HPV -	29	2	N/C	<0.001
		HPV +	0	10		
	M		Cancer			
			-	+		
		HPV -	21	5	N/C	<0.001
		HPV +	0	17		

N/C: could not be calculated because none of the normal controls were HPV +ve

* Calculated from Fisher's exact test

3.1 Human papillomavirus in oral squamous cell carcinoma -UK

The frequency of human papillomavirus DNA among oral lesions is summarised in Table 3.1. An overall HPV-DNA positive proportion of 27/34 (79.4%) was detected in OSCC-UK patients. Demographic characteristics and risk factors for OSCC in the UK patients are summarised in Table 3.2.

3.1.1 Gender

Human papillomavirus-DNA was detected in 10/12 (83.3%) of females compared with 17/22 (77.3%) in males.

3.1.2 Age groups

When HPV-DNA was examined in the different age groups, HPV infection showed a peak in the 70-80 year age group. Human papillomavirus-DNA was detected in 6/8 (75.0%) in the 50-60 year age group, and 4/5 (80.0%) in the 60-70 year age group, reaching a peak of 11/12 (91.7%) in the 70-80 year age group, the frequency then falling to 6/8 (75.0%) in those over 80 years of age. When stratified according to gender and age, all 10 of males aged 70-80 years were HPV infected, while 1/2 (50.0%) of females in this age range were infected with HPV.

3.1.3 Ethnic origins

The results show no statistical difference between the Caucasian and South Asian groups infected with HPV. Human papillomavirus-DNA was detected in 21/26 (80.8%) of Caucasians compared with 6/7 (85.7%) in South East Asian descendents. When stratified according to gender and ethnicity, HPV-DNA was detected in 5/6 (83.3%) of females in both ethnic groups.

However, in contrast, 16/20 (80.0%) of the Caucasian males were HPV infected compared with 1/1 (100.0%) of the South East Asian males.

3.1.4 Association with tobacco, alcohol and Paan

The association between HPV and other major risk factors for OSCC (e.g., tobacco smoking, frequency of smoking, Paan use and alcohol intake) was examined. Human papillomavirus-DNA was detected in 3/3 (100.0%) of current smokers compared with 24/31 (77.4%) of non-smokers. Among the smokers group, HPV-DNA was detected in 2/2 (100.0%) of individuals smoking 10-15/day, and in 1/1 (100.0%) of individuals smoking >20/day. When stratified according to gender and smoking, none of the females in this group were current smokers. HPV-DNA was found in 3/3 (100.0%) of male current smokers. Human papillomavirus-DNA was detected in 10/12 (83.3%) of the female non-smokers group compared with 14/19 (73.7%) of male individuals.

When the use of Paan was examined, HPV-DNA was found in 24/30 (80.0%) of non-Paan users compared with 3/4 (75.0%) of Paan users. The results show no evidence of statistical variation between both groups. When stratified according to gender and current use of Paan, HPV-DNA was detected in 2/2 (100.0%) of females compared with 1/2 (50.0%) in male individuals.

HPV-DNA was detected in 24/30 (80.0%) of non-alcohol drinkers compared with 1/1 (100.0%) of individuals currently consuming 5-20 units of alcohol per week. When stratified to gender and alcohol intake, the results show that HPV was detected in 14/18 (77.8%) of non-alcohol drinking males compared with 10/12 (83.3%) of females within the same group. Among the

male alcohol drinking groups, HPV-DNA found in 1/1 (100.0%) of individuals consuming 5-20 units of alcohol per week, none of the females in the samples were current alcohol consumers. Of note, three male individuals did not report their alcohol consumption and HPV-DNA was detected in 2/3 (66.7%) of these individuals.

3.1.5 Site of oral squamous cell carcinoma samples

Cross tabulation for HPV-DNA frequency and other characteristics are summarised in Table 3.3. Although only small numbers were present in the samples, HPV-DNA was detected in the soft palate, hard palate, anterior two thirds of tongue, lip, commisure and retromolar area, and to a lesser extent, the buccal mucosa, lateral border of the tongue and floor of mouth. HPV-DNA was detected in 5/7 (71.4%) of patients where no data was available regarding the biopsy site. No difference was readily apparent as regards the biopsy site.

3.1.6 Relevant medical history

Human papillomavirus-DNA was detected in 13/15 (86.7%) of patients with no relevant medical history and in 13/18 (72.2%) of patients with second primary OSCC . In addition, HPV-DNA was found in 1/1 (100.0%) of male individuals with candidal infection. When stratified according to gender and medical history HPV was detected in 4/4 (100.0%) of female patients with no relevant medical history, compared with 6/8 (75.0%) of female patients with second primary OSCC. Human papillomavirus DNA was also detected in 9/11 (81.8%) of male patients with no relevant medical history compared with 7/10 (70.0%) of males with second primary OSCC.

male alcohol drinking groups, HPV-DNA found in 1/1 (100.0%) of individuals consuming 5-20 units of alcohol per week, none of the females in the samples were current alcohol consumers. Of note, three male individuals did not report their alcohol consumption and HPV-DNA was detected in 2/3 (66.7%) of these individuals.

3.1.5 Site of oral squamous cell carcinoma samples

Cross tabulation for HPV-DNA frequency and other characteristics are summarised in Table 3.3. Although only small numbers were present in the samples, HPV-DNA was detected in the soft palate, hard palate, anterior two thirds of tongue, lip, commisure and retromolar area, and to a lesser extent, the buccal mucosa, lateral border of the tongue and floor of mouth. HPV-DNA was detected in 5/7 (71.4%) of patients where no data was available regarding the biopsy site. No difference was readily apparent as regards the biopsy site.

3.1.6 Relevant medical history

Human papillomavirus-DNA was detected in 13/15 (86.7%) of patients with no relevant medical history and in 13/18 (72.2%) of patients with second primary OSCC . In addition, HPV-DNA was found in 1/1 (100.0%) of male individuals with candidal infection. When stratified according to gender and medical history HPV was detected in 4/4 (100.0%) of female patients with no relevant medical history, compared with 6/8 (75.0%) of female patients with second primary OSCC. Human papillomavirus DNA was also detected in 9/11 (81.8%) of male patients with no relevant medical history compared with 7/10 (70.0%) of males with second primary OSCC.

male alcohol drinking groups, HPV-DNA found in 1/1 (100.0%) of individuals consuming 5-20 units of alcohol per week, none of the females in the samples were current alcohol consumers. Of note, three male individuals did not report their alcohol consumption and HPV-DNA was detected in 2/3 (66.7%) of these individuals.

3.1.5 Site of oral squamous cell carcinoma samples

Cross tabulation for HPV-DNA frequency and other characteristics are summarised in Table 3.3. Although only small numbers were present in the samples, HPV-DNA was detected in the soft palate, hard palate, anterior two thirds of tongue, lip, commisure and retromolar area, and to a lesser extent, the buccal mucosa, lateral border of the tongue and floor of mouth. HPV-DNA was detected in 5/7 (71.4%) of patients where no data was available regarding the biopsy site. No difference was readily apparent as regards the biopsy site.

3.1.6 Relevant medical history

Human papillomavirus-DNA was detected in 13/15 (86.7%) of patients with no relevant medical history and in 13/18 (72.2%) of patients with second primary OSCC . In addition, HPV-DNA was found in 1/1 (100.0%) of male individuals with candidal infection. When stratified according to gender and medical history HPV was detected in 4/4 (100.0%) of female patients with no relevant medical history, compared with 6/8 (75.0%) of female patients with second primary OSCC. Human papillomavirus DNA was also detected in 9/11 (81.8%) of male patients with no relevant medical history compared with 7/10 (70.0%) of males with second primary OSCC.

The results show that none of the individuals infected with HPV-DNA were receiving any corticosteroid therapy. Human papillomavirus DNA was found in 25/32 (78.1%) of patients with no known immunodeficiency disease compared with 2/2 (100.0%) of patients with systemic immune disease. When stratified according to gender and immune status, HPV was detected in 2/2 (100.0%) of female patients with systemic immune disease.

Human papillomavirus-DNA was detected in 4/6 (66.7%) of patients who had received chemotherapy, in 1/2 (50.0%) of patients who had received radiotherapy and in 22/26 (84.6%) of individuals who had received no therapy. When stratified according to gender and therapy received, HPV was found in 7/8 (87.5%) of female individuals who received no treatment compared with 15/18 (83.3%) of male individuals within the same group. Human papillomavirus DNA was detected in 2/3 (66.7%) of both genders received chemotherapy and in 1/1 (100.0%) in female patients received radiotherapy.

3.1.7 HPV infection in relation to UK-OSCC

The results for HPV infection in relation to OSCC are summarised in Table 3.4. In the sample of people, none of the normal controls was HPV+ve. However, some of the OSCC patients were HPV+ve. Thus, the OSCC patients were highly significantly more likely than normal controls to be HPV+ve in this sample ($P<0.001$, Fisher's exact test). However, since none of the controls was HPV+ve, it was not possible to calculate an odds ratio. Therefore, it was also not possible to control for the effects of any other risk factors in the analysis.

Table 3.5 Detection of human papillomavirus versus demographics and risk factors for oral squamous cell carcinoma of UK patients with proliferative verrucous leukoplakia

Characteristic	- ve No. (%)	+ ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Gender						
Female	2/6(33.3)	4/6 (66.7)				
Male	3/6(50.0)	3/6 (50.0)				
Age (years)						
40-50	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
50-60	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
60-70	1/3(33.3)	2/3(66.7)	0/2(0.0)	2/2(100.0)	1/1(100.0)	0/1(0.0)
70-80	1/6(16.7)	5/6 (83.3)	1/3(33.3)	2/3 (66.7)	0/3(0.0)	3/3 (100.0)
>80	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Ethnicity						
Caucasian	1/5(20.0)	4/5 (80.0)	1/4(25.0)	3/4 (75.0)	0/1(0.0)	1/1 (100.0)
N/A*	4/7(57.1)	3/7 (42.9)	1/2(50.0)	1/2 (50.0)	3/5(60.0)	2/5(40.0)
Tobacco						
None	5/10(50.0)	5/10 (50.0)	2/6 (33.3)	4/6 (66.7)	3/4(75.0)	1/4 (25.0)
current	0/2(0.0)	2/2 (100.0)	-	-	0/2(0.0)	2/2 (100.0)
Smoking						
None	5/10(50.0)	5/10 (50.0)	2/6 (33.3)	4/6 (66.7)	3/4(75.0)	1/4 (25.0)
>20/day	0/2(0.0)	2/2 (100.0)	-	-	0/2(0.0)	2/2 (100.0)
Paan						
None	4/11(36.4)	7/11 (63.6)	2/6 (33.3)	4/6 (66.7)	2/5(40.0)	3/5 (60.0)
Current user	1/1 (100.0)	0/1 (0.0)	-	-	1/1(100.0)	0/1(0.0)
Alcohol						
None	5/11(45.5)	6/11 (54.5)	2/6 (33.3)	4/6 (66.7)	3/5(60.0)	2/5 (40.0)
>20 u/w**	0/1(0.0)	1/1 (100.0)	-	-	0/1(0.0)	1/1 (100.0)
Total	**5/12(41.7)**	**7/ 12(58.3)**	**2/6(33.3)**	**4/6 (66.7)**	**3/6(50.0)**	**3/6 (50.0)**

*N/A= Data not available
**u/w = Units / week

Table 3.6 Cross tabulation for human papillomavirus in UK-proliferative verrucous leukoplakia samples according to biopsy site, relevant medical note, corticosteroids, immune status, and therapies

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%))	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Biopsy site						
Buccal mucosa	1/4(25.0)	3/4(75.0)	1/4(25.0)	3/4(75.0)		
Lt.of tongue*	1/2(50.0)	1/2(50.0)	-	-	1/2(50.0)	1/2(50.0)
Floor of mouth	1/2(50.0)	1/2(50.0)	-	-	1/2(50.0)	1/2(50.0)
Hard palate	0/1(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	-	-
Ant. 2/3 of tongue**	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Lip	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Retromolar	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Medical note						
None	2/3(66.7)	1/3(33.3)	1/2(50.0)	1/2(50.0)	1/1(100.0)	0/1(0.0)
SCC***	2/6(33.3)	4/6(66.7)	1/3(33.3)	2/3(66.7)	1/3(33.3)	2/3(66.7)
Candidal infection	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Penicillin allergy	1/2(50.0)	1/2(50.0)	0/1(0.0)	1/1(100.0)	1/1(100.0)	0/1(0.0)
Corticosteroids						
None	5/12(41.7)	7/12(58.3)	2/6(33.3)	4/6(66.7)	3/6(50.0)	3/6(50.0)
Immune status						
None	5/12(41.7)	7/12(58.3)	2/6(33.3)	4/6(66.7)	3/6(50.0)	3/6(50.0)
Therapies						
None	5/11(54.5)	6/11(45.5)	2/5(40.0)	3/5(60.0)	3/6(50.0)	3/6(50.0)
Chemotherapy	0/1(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	-	-
Total	**5/12(41.7)**	**7/12(58.3)**	**2/6(33.3)**	**4/6(66.7)**	**3/6(50.0)**	**3/6 (50.0)**

* Lt. of tongue= lateral border of the tongue
** Ant. 2/3 of tongue= anterior 2/3 of tongue
*** SCC= Second-primary Squamous Cell Carcinoma

Table 3.7 Human papillomavirus infection in relation to UK-proliferative verrucous leukoplakia samples

UK	Gender			PVL -	PVL +	Odds Ratio (95 % CI)	P-value*
PVL							
	All						
		HPV	-	50	5	N/C	<0.001
			+	0	7		
	F						
		HPV	-	29	2	N/C	<0.001
			+	0	4		
	M						
		HPV	-	21	3	N/C	<0.001
			+	0	3		

N/C: Could not be calculated because none of the normal controls was HPV+ve

* Calculated from Fisher's exact test

3.2 Human papillomavirus in proliferative verrucous leukoplakia-UK

The frequency of human papillomavirus DNA among oral lesions is summarised in Table 3.1. An overall HPV-DNA positive proportion of 7/12 (58.3%) was detected in PVL-UK patients. Demographic characteristics and risk factors for PVL in the UK patients are summarised in Table 3.5.

3.2.1 Gender

Human papillomavirus-DNA was detected in 4/6 (66.7%) of females compared with 3/6 (50.0%) in males.

3.2.2 Age groups

Human papillomavirus-DNA detection was 2/3 (66.7%) in the 60-70 year age group and 5/6 (83.3%) in the 70-80 year age group. When stratified according to age and gender, HPV-DNA was detected in 2/2 (100.0%) of females aged 60-70 years, and in 2/3 (66.7%) of females aged 70-80 years compared with 3/3 (100.0%) of males within the same age group.

3.2.3 Ethnic origins

Human papillomavirus-DNA was detected in 4/5 (80.0%) of Caucasians compared with 3/7 (42.9%) in patients where ethnicity was not recorded. When further stratified according to gender and ethnicity, HPV-DNA was detected in 3/4 (75.0%) of Caucasian females compared with 1/1 (100.0%) of males of the same ethnicity.

3.2.4 Associations with tobacco/alcohol and Paan

The association between HPV and other major risk factors for PVL (e.g., tobacco smoking, frequency of smoking, Paan use and alcohol intake)

was examined. Human papillomavirus-DNA was found in 2/2 (100.0%) of current smokers compared with 5/10 (50.0%) of non-smokers. Among the smokers group, HPV-DNA was detected in 2/2 (100.0%) of individuals smoking >20/day. When further stratified according to gender and smoking, none of the females in this group were current smokers. Human papillomavirus-DNA was found in 1/4 (25.0%) of male non-smokers compared with 2/2 (100.0%) of male individuals smoking >20/day group.

When the use of Paan was examined, HPV-DNA was found only in 7/11 (63.6%) of non-Paan users. When stratified according to gender and use of Paan, HPV-DNA was found in non-Paan users in 4/6 (66.7%) of females compared with 3/5 (60.0%) of male individuals not using Paan.

Human papillomavirus-DNA was detected in 6/11 (54.5%) of non-alcohol drinkers compared with 1/1 (100.0%) of individuals currently consuming >20 units per week. When stratified according to gender and alcohol intake, HPV-DNA was found 4/6 (66.7%) of female non-alcohol drinkers. Human papillomavirus-DNA was detected in 1/1 (100.0%) of male individuals consuming >20 units per week compared with 2/5 (40.0%) of male non-alcohol drinkers.

3.2.5 Site of proliferative verrucous leukoplakia samples

Cross tabulation for HPV-DNA frequency and other characteristics are summarised in Table 3.6. Human papillomavirus-DNA was detected in the buccal mucosa, lateral border of the tongue, floor of mouth, hard palate and in the anterior 2/3 of the tongue. When stratified according to gender and biopsy site, no difference was readily apparent as regards the biopsy site.

3.2.6 Relevant medical history

Human papillomavirus-DNA was detected in 1/3 (33.3%) of patients with no relevant medical history. In addition, HPV-DNA was found in 4/6 (66.7%) of patients with second primary OSCC, in 1/1 (100.0%) of patients with candidal infection and in 1/2 (50.0%) of patients allergic to penicillin. When stratified according to gender and medical history, HPV-DNA was found in 1/2 (50.0%) of females with no relevant medical history, in 2/3 (66.7%) with second primary OSCC, and in 1/1 (100.0%) of patients allergic to penicillin. Human papillomavirus-DNA was found in 2/3 (66.7%) of male patients with second primary OSCC, and in 1/1 (100.0%) of those with previous candidal infection.

The results show that none of the individuals infected with HPV-DNA had received any cortico-steroid therapy or had any known immune disease. In patients receiving chemotherapy, HPV-DNA was found in 1/1 (100.0%) of female individuals compared with 3/5 (60.0%) of female individual received no therapy. Human papillomavirus-DNA was detected in 3/6 (50.0%) of male individuals recieved no therapy.

3.2.7 HPV infection in relation to UK-PVL

The results for HPV infection in relation to PVL are summarised in Table 3.7. In the sample of people, none of the normal controls was HPV+ve. However, some of the PVL patients were HPV+ve. Thus the PVL patients were highly significantly more likely than normal controls to be HPV+ve in this sample ($P<0.001$, Fisher's exact test). However, since none of the controls were HPV+ve, it was not possible to calculate an odds ratio.

Therefore, it was also not possible to control for the effects of any other risk factors in this analysis.

Table 3.8 Detection of human papillomavirus versus demographics and risk factors for oral squamous cell carcinoma of UK patients with oral epithelial dysplasia

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Gender						
Female	12/22(54.5)	10/22(45.5)				
Male	14/23(60.9)	9/23 (39.1)				
Age (years)						
20-30	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)
30-40	2/2(100.0)	0/2(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)
40-50	2/3(66.7)	1/3 (33.3)	-	-	1/2(50.0)	1//2 (50.0)
50-60	10/16(62.5)	6/16 (37.5)	4/6(66.7)	2/6(33.3)	6/10(60.0)	4/10 (40.0)
60-70	6/14(42.9)	8/14 (57.4)	2/7(28.6)	5/7(71.4)	4/7(57.1)	3/7 (42.9)
70-80	5/9(55.6)	4/9 (44.4)	4/7(57.1)	3/7(42.9)	1/2(50.0)	1/2(50.0)
Ethnicity						
Caucasian	20/30(66.7)	10/30 (33.3)	10/14(71.4)	4/14 (28.6)	10/16(62.5)	6/16(37.5)
SEA*	2/6(33.3)	4/6 (66.7)	1/4(25.0)	3/4 (75.0)	1/2 (50.0)	1/2(50.0)
N/A**	4/9(44.4)	5/9 (55.6)	1/4(25.0)	3/4 (75.0)	3/5(60.0)	2/5(40.0)
Tobacco						
None	13/24(54.2)	11/24 (45.8)	7/15(46.7)	8/15(53.3)	6/9(66.7)	3/9(33.3)
Ex-smoker	1/3(33.3)	2/3 (66.7)	-	-	1/3(33.3)	2/3(66.7)
Current	12/18(66.7)	6/18 (33.3)	5/7(71.4)	2/7(28.6)	7/11(63.6)	4/11(36.4)
Smoking						
None	13/24(54.2)	11/24 (45.8)	7/15(46.7)	8/15(53.3)	6/9(66.7)	3/9(33.3)
10-15/day	6/12(50.0)	6/12 (50.0)	2/4(50.0)	2/4(50.0)	4/8(50.0)	4/8 (50.0)
>20/day	7/9(77.8)	2/9 (22.2)	3/3(100.0)	0/3(0.0)	4/6(66.7)	2/6(33.3)
Paan						
None	24/40(60.0)	16/40 (40.0)	11/19(57.9)	8/19(42.1)	13/21(61.9)	8/21(38.1)
Current	2/5(40.0)	3/5 (60.0)	1/3(33.3)	2/3(66.6)	1/2(50.0)	1/2(50.0)
Alcohol						
None	13/23(56.5)	10/23 (43.5)	5/11(45.5)	6/11(54.5)	8/12(66.7)	4/12(33.3)
<5 u/w***	3/4(75.0)	1/4 (25.0)	2/3(66.7)	1/3 (33.3)	1/1(100.0)	0/1(0.0)
5-20 u/w	0/3(0.0)	3/3 (100.0)	0/1(0.0)	1/1 (100.0)	0/2(0.0)	2/2(100.0)
> 20 u/w	7/12(58.3)	5/12 (41.7)	4/6(66.6)	2/6 (33.3)	3/6(50.0)	3/6(50.0)
N/A	3/3(100.0)	0/3(0.0)	1/1(100.0)	0/1(0.0)	2/2(100.0)	0/2(0.0)
Total	**26/45(57.8)**	**19/45 (42.2)**	**12/22(54.5)**	**10/22(45.5)**	**14/23(60.9)**	**9/23 (39.1)**

*SEA= South East Asian
**N/A= Data not available
***u/w= units per week

Table 3.9 Cross tabulation for human papillomavirus in UK-oral epithelial dysplasia samples according to biopsy site, relevant medical note, corticosteroids, immune status, and therapies.

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Biopsy site						
Buccal mucosa	4/8(50.0)	4/8(50.0)	1/3(33.3)	2/3(66.7)	3/5(60.0)	2/5(40.0)
Lt. of tongue*	7/12(58.3)	5/12(41.7)	2/6(33.3)	4/6(66.7)	5/6(83.3)	1/6(16.7)
Floor of mouth	2/3(66.7)	1/3(33.3)	1/2(50.0)	1/2(50.0)	1/1(100.0)	0/1(0.0)
Hard palate	0/2(0.0)	2/2 (100.0)	-	-	0/2(0.0)	2/2(100.0)
Soft palate	0/2(0.0)	2/2 (100.0)	-	-	0/2(0.0)	2/2(100.0)
Ant. 2/3 of tongue**	3/4(75.0)	1/4 (25.0)	3/4(75.0)	1/4(25.0)	-	-
Lip	1/2(50.0)	1/2 (50.0)	1/1(100.0)	0/1(0.0)	0/1(0.0)	1/1(100.0)
Commisure	8/9(88.9)	1/9 (11.1)	3/3(100.0)	0/3(0.0)	5/6(83.3)	1/6(16.7)
Retromolar	0/1(0.0)	1/1 (100.0)	0/1(0.0)	1/1(100.0)	-	-
Salivary	0/1(0.0)	1/1 (100.0)	0/1(0.0)	1/1(100.0)	-	-
Attached gingiva	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Relevant medical note						
None	13/20(65.0)	7/20 (35.0)	7/11(63.6)	4/11(36.4)	6/9(66.7)	3/9(33.3)
SCC***	2/7(28.6)	5/7 (71.4)	0/2(0.0)	2/2(100.0)	2/5(40.0)	3/5(60.0)
Candidal infection	4/5(80.0)	1/5(20.0)	2/2(100.0)	0/2(0.0)	2/3(66.7)	1/3(33.3)
Submucous fibrosis	1/4(25.0)	3/4 (75.0)	0/2(0.0)	2/2(100.0)	1/2(50.0)	1/2 (50.0)
HRT****	3/5(60.0)	2/5(40.0)	3/5(60.0)	2/5(40.0)	-	-
Arthritis	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Asthma	2/2(100.0)	0/2(0.0)	-	-	2/2(100.0)	0/2(0.0)
Diabetes mellitus	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Corticosteroids						
None	26/45(57.8)	19/45(42.2)	12/22(54.5)	10/22(45.5)	14/23(60.9)	9/23(39.1)
Immune status						
None	25/43(58.1)	18/43(41.9)	11/21(52.4)	10/21(47.6)	14/22(63.6)	8/22(36.4)
Systemic	1/2(50.0)	1/2(50.0)	1/1(100.0)	0/1(0.0)	0/1(0.0)	1/1(100.0)
Therapies						
None	24/41(58.5)	17/41(41.5)	10/18(55.6)	8/18(44.4)	14/23(60.9)	9/23(39.1)
Chemotherapy	2/4(50.0)	2/4 (50.0)	2/4(50.0)	2/4(50.0)	-	-
Total	**26/45(57.8)**	**19/45(42.2)**	**12/22(54.5)**	**10/22(45.5)**	**14/23(60.9)**	**9/23 (39.1)**

* Lt. of tongue= lateral border of the tongue
** Ant. 2/3 of tongue= anterior 2/3 of tongue
*** SCC= Second-primary Squamous Cell Carcinoma
****HRT=Hormone replacement therapy

Table 3.10 Human papillomavirus infection in relation to UK-oral epithelial dysplasia samples

UK OED	Gender				Odds Ratio (95% CI)	P-value*
	All		OED -	OED +		
		HPV -	50	26	N/C	<0.001
		HPV +	0	19		
	F		OED -	OED +		
		HPV -	29	12	N/C	<0.001
		HPV +	0	10		
	M		OED -	OED +		
		HPV -	21	14	N/C	<0.001
		HPV +	0	9		

N/C: could not be calculated because none of the normal controls were HPV +ve

* Calculated from Fisher's exact test

3.3 Human papillomavirus in oral epithelial dysplasia-UK

The frequency of human papillomavirus DNA among oral lesions is summarised in Table 3.1. An overall HPV-DNA positive proportion of 19/45 (42.2%) was detected in OED-UK patients. Demographic characteristics and risk factors for OED in the UK patients are summarised in Table 3.8.

3.3.1 Gender

Human papillomavirus-DNA was detected in 10/22 (45.5%) of females compared with 9/23 (39.1%) in males.

3.3.2 Age groups

When HPV-DNA was examined in different age groups the proportion was 1/3 (33.3%) in the 40-50 year age group, 6/16 (37.5%) in the 50-60 year age group, 8/14 (57.4%) in the 60-70 year age group and 4/9 (44.4%) in the 70-80 year age group. When stratified according to gender and age groups, HPV-DNA was found in 2/6 (33.3%) of the female group aged 50-60 years compared with 4/10 (40.0%) of males within the same age group. Human papillomavirus-DNA was detected in 5/7 (71.4%) of the female group aged 60-70 years compared with 3/7 (42.9%) of males within the same age group. In addition, HPV-DNA was found in 3/7 (42.9%) of the female aged 70-80 years compared with 1/2 (50.0%) of males within the same age group.

3.3.3 Ethnic origins

Human papillomavirus-DNA was detected in 10/30 (33.3%) of Caucasians compared with 4/6 (66.7%) in South East Asians. When stratified according to gender and ethnicity, HPV-DNA was found in 4/14 (28.6%) of female Caucasians compared with 6/16 (37.5%) of the male Caucasians.

However, in South East Asians HPV-DNA was found in 3/4 (75.0%) of females compared with 1/2 (50.0%) of males. HPV-DNA was found in 5/9 (55.6%) of patients with no recorded ethnicity.

3.3.4 Associations with tobacco, alcohol and Paan

The association between HPV and other major risk factors for PVL (e.g. tobacco smoking, frequency of smoking, Paan use and alcohol intake) was examined. Human papillomavirus-DNA was found in 6/18 (33.3%) of current smokers compared with 11/24 (45.8%) in non-smokers. In addition, HPV was positive in 2/3 (66.7%) of ex-smokers. Among the smokers group, HPV-DNA was detected in 6/12 (50.0%) of individuals smoking 10-15/day and in 2/9 (22.2%) of individuals smoking >20/day. When further stratified according to gender and smoking, HPV-DNA was detected in none of the female ex-smoking group compared with 2/3 (66.7%) of male ex-smokers. Human papillomavirus-DNA was found in 4/8 (50.0%) of male smoking 10-15/day compared with 2/6 (33.3%) of male smoking >20 /day.

When the use of Paan was examined, HPV-DNA was found in 16/40 (40.0%) of non-Paan users compared with 2/5 (40.0%) of current Paan users. When stratified according to gender and use of Paan, HPV-DNA was found in 8/19 (42.1%) of female non-Paan users compared with 8/21 (38.1%) of male individuals. In current Paan users, HPV-DNA was found in 2/3 (66.7%) of females compared with 1/2 (50.0%) of male individuals currently using Paan.

Human papillomavirus-DNA was detected in 10/23 (43.5%) of non-alcohol drinkers. However, HPV-DNA was detected in 1/4 (25.0%) of individuals currently consuming < 5 units per week, in 3/3 (100.0%) of

individuals currently consuming 5-20 units per week and in 5/12 (41.7%) of individuals consuming >20 units per week. When stratified according to gender and alcohol intake, HPV-DNA was found 6/11 (54.5%) of female non-alcohol drinkers compared with 4/12 (33.3%) of male individuals. Human papillomavirus-DNA was detected in all two male individuals consuming 5-20 units per week compared with 1/1 (100.0%) of female individuals. In addition, HPV-DNA was detected in 3/6 (50.0%) of male individuals consuming >20 units per week compared with 2/6 (33.3%) of females within the same group.

3.3.5 Site of oral epithelial dysplasia samples

Cross tabulation for HPV-DNA frequency and other characteristics are summarised in Table 3.9. Human papillomavirus-DNA was detected in the buccal mucosa, lateral border of the tongue, soft palate, hard palate, and to a lesser extent, anterior two thirds of tongue, lip, commisure, retromolar area, and floor of mouth. No difference was readily apparent as regards the biopsy site and HPV infection. Similar proportions were found in both genders.

3.3.6 Relevant medical history

Human papillomavirus-DNA was detected in 7/20 (35.0%) of patients with no relevant medical history and in 5/7 (71.4%) of patients with second primary OSCC. In addition, HPV-DNA was found in 1/5 (20.0%) of individuals with candidal infection, in 3/4 (75.0%) of patients with submucous fibrosis, in 2/5 (40.0%) of female patients receiving HRT and in 1/1 (100.0%) of male individuals with a history of arthritis. When stratified according to gender and medical history, HPV was detected in 4/11 (36.4%) of females with no relevant medical history compared with 3/9 (33.3%) of male individuals within

same group, and in 2/2 (100.0%) of females with second primary compared with 3/5 (60.0%) of male individuals within the same group. In addition, HPV was positive in 2/2 (100.0%) of females with submucous fibrosis compared with 1/2(50.0%) of males with the same disease.

The results show that none of the individuals infected with HPV-DNA had received any corticosteroid therapy. Human papillomavirus was found in 18/43 (41.9%) of patients of both genders without any known immune disease compared with 1/1 (100.0%) of males with systemic immune disease.

Human papillomavirus-DNA was detected in 2/4 (50.0%) of patients received chemotherapy compared with 17/41 (41.5%) of patients who had received no such treatment. When stratified according to gender and receiving chemotherapy, HPV was found in 2/4 (50.0%) of female individuals.

3.3.7 HPV infection in relation to UK-OED

The results for HPV infection in relation to OED are summarised in Table 3.10. In the sample of people, none of the normal controls was HPV+ve. However, some of the OED patients were HPV+ve. Thus the OED patients were highly significantly more likely than normal controls to be HPV+ve in this sample, ($P<.0001$, Fisher's exact test). However, since none of the controls was HPV+ve, it was not possible to calculate an odds rsatio. Therefore, it was also not possible to control for the effects of any other risk factors in this analysis.

Table 3.11 Detection of human papillomavirus versus demographics and risk factors for oral squamous cell carcinoma of UK patients with oral lichen planus

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Gender						
Female	10/14(71.4)	4/14(28.6)				
Male	11/16(68.8)	5/16(31.3)				
Age (years)						
40-50	5/7(71.4)	2/7(28.6)	3/4(75.0)	1/4(25.0)	2/3(66.7)	1/3(33.3)
50-60	6/10(60.0)	4/10(40.0)	3/5(40.0)	2/5(40.0)	3/5(60.0)	2/5(40.0)
60-70	5/8(62.5)	3/8(37.5)	2/3(66.7)	1/3(33.3)	3/5(60.0)	2/5(40.0)
70-80	5/5(100.0)	0/5(0.0)	2/2(100.0)	0/2(0.0)	3/3(100.0)	0/3(0.0)
Ethnicity						
Caucasian	12/16(75.0)	4/16(25.0)	6/8(75.0)	2/8(25.0)	6/8(75.0)	2/8(25.0)
SEA*	5/5(100.0)	0/5(0.0)	3/3(100.0)	0/3(0.0)	2/2(100.0)	0/2(0.0)
N/A**	4/9(44.4)	5/9(55.6)	1/3(33.3)	2/3(66.7)	3/6(50.0)	3/6(50.0)
Tobacco						
None	19/26(73.1)	7/26(26.9)	9/13(69.2)	4/13(30.8)	10/13(76.9)	3/13(23.1)
Ex-smoker	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Current	2/3(66.7)	1/3(33.3)	1/1(100.0)	0/1(0.0)	1/2(50.0)	1/2(50.0)
Smoking						
None	19/26(73.1)	7/26(26.9)	9/13(69.2)	4/13 (30.8)	10/13(76.9)	3/13(23.1)
<5 /day	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
10-15/day	0/2(0.0)	2/2(100.0)	-	-	0/2(0.0)	2/2(100.0)
>20/day	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Paan						
None	20/29(69.0)	9/29(31.0)	9/13(69.2)	4/13(30.8)	11/16(68.8)	5/16(31.3)
Current	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Alcohol						
None	16/23(69.6)	7/23(30.4)	8/12(66.7)	4/12(33.3)	8/11(72.7)	3/11(27.3)
5-20u/w ***	1/2(50.0)	1/2(50.0)	1/1(100.0)	0/1(0.0)	0/1(0.0)	1/1(100.0)
>20 u/ w	1/2(50.0)	1/2(50.0)	-	-	1/2(50.0)	1/2(50.0)
N/A	3/3(100.0)	0/3(0.0)	1/1(100.0)	0/1(0.0)	2/2(100.0)	0/2(0.0)
Total	**21/30(70.0)**	**9/30(30.0)**	**10/14(71.4)**	**4/14(28.6)**	**11/16(68.8)**	**5/16(31.3)**

*SEA=South East Asian
**N/A= Data not available
*** u/w = units per week

Table 3.12 Cross tabulation for human papillomavirus in UK-oral lichen planus samples according to biopsy site, relevant medical note, corticosteroids, immune status, and therapies

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Biopsy site						
Buccal mucosa	18/25(72.0)	7/25(28.0)	9/11(81.8)	2/11(18.2)	9/14(64.2)	5/14(35.7)
Lt. of tongue*	2/3(66.7)	1/3 (33.3)	1/2(50.0)	1/2(50.0)	1/1(100.0)	0/1(0.0)
Attached gingiva	1/2(50.0)	1/2(50.0)	0/1(0.0)	1/1(100.0)	1/1(100.0)	0/1(0.0)
Medical note						
None	10/17(58.8)	7/17(41.2)	2/5(40.0)	3/5(60.0)	8/12(66.7)	4/12(33.3)
Cutaneous disease	3/4(75.0)	1/4(25.0)	2/2(100.0)	0/2(0.0)	1/2(50.0)	1/2(50.0)
Diabetes mellitus	1/2(50.0)	1/2(50.0)	1/2 (50.0)	1/2(50.0)	-	-
HRT**	2/2(100.0)	0/2(0.0)	2/2(100.0)	0/2(0.0)	-	-
Asthma	2/2(100.0)	0/2(0.0)	2/2(100.0)	0/2(0.0)	-	-
Candida	3/3(100.0)	0/3(0.0)	1/1(100.0)	0/1(0.0)	2/2(100.0)	0/2(0.0)
Corticosteroids						
None	18/26(69.2)	8/26(30.8)	8/11(72.7)	3/11(27.3)	10/15(66.7)	5/15(33.3)
Topical	0/1(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	-	-
Systemic	3/3(100.0)	0/3(0.0)	2/2(100.0)	0/2(0.0)	1/1(100.0)	0/1(0.0)
Immune status						
None	13/21(61.9)	8/21(38.1)	5/9(55.6)	4/9(44.4)	8/12(66.7)	4/12(33.3)
Drugs	4/5(80.0)	1/5 (20.0)	2/2(100.0)	0/2(0.0)	1/2 (50.0)	1/2(50.0)
Systematic	4/4(100.0)	0/4(0.0)	3/3(100.0)	0/3(0.0)	2/2(100.0)	0/2(0.0)
Therapies						
None	21/30(70.0)	9//30(30.0)	10/14(71.4)	4/14(28.6)	11/16(68.8)	5/16(31.3)
Total	**21/30(70.0)**	**9/30(30.0)**	**10/14(71.4)**	**4/14(28.6)**	**11/16(68.8)**	**5/16(31.3)**

* Lt. of tongue= lateral border of the tongue
**HRT=Hormone replacement therapy

Table 3.13 Human papillomavirus infection in relation to UK-oral lichen planus samples

UK	Gender				Odds Ratio (95% CI)	P-value*
OLP						
	ALL			OLP		
				-	+	
		HPV	-	50	21	N/C	<0.001
			+	0	9		
	F			OLP		
				-	+	
		HPV	-	29	10	N/C	<0.001
			+	0	4		
	M			OLP		
				-	+	
		HPV	-	21	11	N/C	<0.001
			+	0	5		

N/C: could not be calculated because none of the normal controls were HPV +ve

* Calculated from Fisher's exact test

3.4 Human papillomavirus in oral lichen planus - UK

The frequency of human papillomavirus DNA among oral lesions is summarised in Table 3.1 An overall HPV-DNA positive proportion of 9/30 (30.0%) was detected in OLP-UK patients. Demographic characteristics and risk factors for OLP in the UK pateints are summarised in Table 3.11.

3.4.1 Gender

Human papillomavirus-DNA was detected in 4/14 (28.6%) of females compared with 5/16 (31.3%) of males.

3.4.2 Age groups

When HPV-DNA was examined in different age groups, HPV-DNA was detected in 2/7 (28.6%) in the 40-50 year age group, 4/10 (40.0%) of the 50-60 year age group and 3/8 (37.5%) of the 60-70 year age group. When stratified according to gender and age groups, HPV-DNA was found in 1/4 (25.0%) of the female 40-50 years age group compared with 1/3 (33.3%) of males within the same age group, HPV-DNA was found in 2/5 (40.0%) of the 50-60 year age group in both genders. In addition, HPV-DNA was found in 1/3 (33.3%) of females aged 60-70 years compared with 2/5 (40.0%) of males within the same age group.

3.4.3 Ethnic origins

Human papillomavirus-DNA was detected in 4/16 (25.0%) of Caucasians compared with 4/9 (44.4%) in patients with no recorded ethnicity. When stratified according to gender and ethnicity, HPV-DNA was found in 2/8 (25.0%) of both genders in Caucasian. However, HPV-DNA was found in

2/3 (66.7%) of the females with no recorded ethnicity compared with 3/6 (50.0%) of the males within the same group.

3.4.4 Associations with tobacco, alcohol and Paan

The association between HPV and other major risk factors for OLP (e.g., tobacco smoking, frequency of smoking, Paan use and alcohol intake) was examined. HPV-DNA was found in 1/3 (33.3%) of current smokers compared with 7/26 (26.9%) of non-smokers. In addition, HPV was positive in 1/1 (100.0%) of ex-smokers. Among the smokers group, HPV-DNA was detected in both 2/2 (100%) individuals smoking 10-15/day. When further stratified according to gender and smoking, none of the females in this group were ex-smokers while 1/1 (100.0%) of HPV positive males was ex-smoker.

When the use of Paan was examined, HPV-DNA was found in 9/29 (31.0%) of non-Paan users. When stratified according to gender and use of Paan, HPV-DNA was found in 4/13 (30.8%) of non-Paan users female individuals compared with 5/16 (31.3%) of non-Paaan users males.

Human papillomavirus-DNA was detected in 7/23 (30.4%) of non-alcohol drinkers. However, HPV-DNA was detected in 1/2 (50.0%) of both groups of individuals currently consuming 5-20 units per week and of individuals consuming >20 units per week. When stratified according to gender and alcohol intake, human papillomavirus-DNA was found in 4/12 (33.3%) of non-alcohol drinking females compared with 3/11 (27.3%) of male individuals within the same group. Among the male alcohol drinking group, HPV-DNA was detected in 1/1 (100.0%) of male consuming 5-20 units per week compared with 1/2 (50.0%) of males consuming >20 units per week.

3.4.5 Site of oral lichen planus samples

Cross tabulation for HPV-DNA frequency and other characteristics are summarised in Table 3.12. Human papillomavirus-DNA was detected in the buccal mucosa, lateral border of the tongue and attached gingiva. no differences in HPV infection were readily apparent as regards the biopsy site. When further stratified according to gender and biopsy site, HPV-DNA was found in the buccal mucosa of 5/14 (35.7%) of males compared with 2/11 (18.2%) of females within the same group.

3.4.6 Relevant medical history

Human papillomavirus-DNA was detected in 7/17 (41.2%) of patients with no relevant medical history, in 1/2 (50.0%) of male patients with a skin lesion and in 1/2 (50.0%) of diabetic female patients. The results show that HPV-DNA was found in 8/26 (30.8%) of patients who received no cortico-steroids compared with 1/1 (100.0%) female receiving topical steroids. Human papillomavirus was found in 8/21 (38.1%) of patients without any immune disease compared with 1/2 (50.0%) of male patients receiving immunosuppressive drugs. None of the patients' detected positive for HPV-DNA had received chemotherapy or radiotherapy.

3.4.7 HPV infection in relation to OLP

The results for HPV infection in relation to OLP are summarised in Table 3.13. In the sample of people, none of the normal controls was HPV+ve. However, some of the OLP patients were HPV+ve. Thus the OLP patients were highly significantly more likely to be HPV+ve than normal controls in this sample. ($P<0.001$, Fisher's exact test). However, since none

of the controls were HPV+ve it was not possible to calculate an odds ratio. Therefore, it was also not possible to control for the effects of any other risk factors in this analysis.

Table 3.14 Detection of human papillomavirus versus demographics and risk factors for oral squamous cell carcinoma of UK patients with human immunodeficiency virus

Characteristic		-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Gender							
	Female	2/3 (66.7)	1/3(33.3)				
	Male	14/22(63.6)	8/22(36.4)				
Age (years)							
	30-40	6/8(75.0)	2/8(25.0)	1/2(50.0)	1/2(50.0)	5/6(83.3)	1/6 (16.7)
	40-50	8/13(61.5)	5/13(38.5)	-	-	8/13(61.5)	5/13(38.5)
	50-60	2/4(50.0)	2/4(50.0)	1/1(100.0)	0/1 (0.0)	1/3(33.3)	2/3 (66.7)
Ethnicity							
	Caucasian	15/22(68.2)	7/22(31.8)	1/2(50.0)	1/2(50.0)	14/20(70.0)	6/20(30.0)
	African	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
	SEA*	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1 (0.0)	-	-
	N/A**	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Tobacco							
	None	9/18(50.0)	9/18(50.0)	2/3(66.7)	1/3(33.3)	7/15(46.7)	8/15(53.3)
	Current	7/7(100.0)	0/7(0.0)	-	-	7/7(100.0)	0/7(0.0)
Smoking							
	None	9/18(50.0)	9/18(50.0)	2/3(66.7)	1/3(33.3)	7/15(46.7)	8/15(53.3)
	>20/day	7/7(100.0)	0/7(0.0)	-	-	7/7(100.0)	0/7(0.0)
Paan							
	None	16/25(64.0)	9/25(36.0)	2/3(66.7)	1/3(33.3)	14/22(63.6)	8/22(36.4)
Alcohol							
	None	15/25(60.0)	9/24(37.5)	2/3(66.7)	1/3(33.3)	13/22(59.1)	8/21(38.1)
	>20 u/w***	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Total		**16/25(64.0)**	**9/25(36.0)**	**2/3(66.7)**	**1/3(33.3)**	**14/22(63.6)**	**8/22(36.4)**

* SEA=South East Asian
**N/A= Data not available
*** u/w= Units per week

Table 3.15 Cross tabulation for human papillomavirus in human immunodeficiency virus samples according to biopsy site, relevant medical note, corticosteroids, immune status, and therapies

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Biopsy site						
Buccal mucosa	3/4(75.0)	1/4(25.0)	-	-	3/4(75.0)	1/4(25.0)
Lt. of tongue*	0/2(0.0)	2/2(100.0)	-	-	0/2(0.0)	2/2(100.0)
Attached gingiva	0/2(0.0)	2/2(100.0)	-	-	0/2(0.0)	2/2(100.0)
Hard palate	3/5(60.0)	2/5(40.0)	1/2(50.0)	1/2(50.0)	2/3(66.7)	1/3(33.3)
Ant. 2/3 of tongue**	0/1(0.0)	1/1(100.0)			0/1(0.0)	1/1(100.0)
Lip	6/6(100.0)	0/6(0.0)	1/1(100.0)	0/1(0.0)	5/5(100.0)	0/5(0.0)
Commisure	3/3(100.0)	0/3(0.0)	-	-	3/3(100.0)	0/3(0.0)
Retromolar	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Other sites***	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Relevant medical note						
None	12/20(60.0)	8/20(40.0)	2/3(66.7)	1/3(33.3)	10/17(58.8)	7/17(41.2)
Candidal infection	3/4(75.0)	1/4(25.0)	-	-	3/4 (75.0)	1/4 (25.0)
Cutaneous disease	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Corticosteroids						
None	14/18(77.8)	4/18(22.2)	1/2 (50.0)	1/2(50.0)	13/16(81.3)	3/16(18.8)
Systemic	2/7(28.6)	5/7(71.4)	1/1(100.0)	0/1(0.0)	1/6(16.7)	5/6(31.3)
Immune status						
None	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Systemic	15/24(62.5)	9/24(37.5)	2/3(66.7)	1/3(33.3)	13/21(61.9)	8/21(38.1)
Therapies						
None	10/19(52.6)	9/19(47.4)	2/3(66.7)	1/3(33.3)	8/16(50.0)	8/16(50.0)
Triple therapy	6/6(100.0)	0/6(0.0)	-	-	6/6(100.0)	0/6(0.0)
Total	**16/25(64.0)**	**9/25(36.0)**	**2/3(66.7)**	**1/3(33.3)**	**14/22(63.6)**	**8/22(36.4)**

* Lt. of tongue= lateral border of the tongue
** Ant. 2/3 of tongue= anterior 2/3 of tongue
*** Other sites= Data not available

Table 3.16 Human papillomavirus infection in relation to UK- human immunodeficiency virus samples

UK	Gender			HIV -	HIV +	Odds Ratio (95% CI)	P-value*
HIV							
	ALL						
		HPV	-	50	16	N/C	<0.001
			+	0	9		
	F						
		HPV	-	29	2	N/C	<0.001
			+	0	1		
	M						
		HPV	-	21	14	N/C	<0.001
			+	0	8		

N/C: could not be calculated because none of the normal controls were HPV +ve
* Calculated from Fisher's exact test

3.5Human papillomavirus in Human immunodeficiency virus individuals

The frequency of human papillomavirus DNA among oral lesions is summarised in Table 3.1. An overall HPV-DNA positive proportion of 9/25 (36.0%) was detected in HIV-UK patients. Demographic characteristics and risk factors for HIV in the UK patients are summarised in Table 3.14.

3.5.1 Gender

Human papillomavirus-DNA was detected in 1/3 (33.3%) of females compared with 8/22 (36.4%) in males.

3.5.2 Age groups

When HPV-DNA was examined in different age groups, the proportion was 2/8 (25.0%) in the 30-40 year age group, 5/13 (38.5%) in the 40-50 year age group and 2/4 (50.0%) in the 50-60 year age group. When stratified according to gender and age, HPV-DNA was found in 1/2 (50.0%) of the female group aged 30-40 years compared with 1/6 (16.7%) of the males within the same age group. Human papillomavirus-DNA was detected in 5/13 (38.5%) of the male group aged 40-50 years and in 2/3 (66.7%) of the male group aged 50-60 years.

3.5.3 Ethnic origins

Human papillomavirus-DNA was detected in 7/22 (31.8%) of Caucasians compared with 1/1 (100.0%) African. When stratified according to gender and ethnicity, HPV-DNA was found in 1/2 (50.0%) of Caucasian females compared with 6/20 (30.0%) of the Caucasian males. HPV-DNA was found in 1/1 (100.0%) of male patient with no recorded ethnicity.

3.5.4 Association with tobacco, alcohol and Paan

The association between HPV and other major risk factors for HIV (e.g., tobacco smoking, frequency of smoking, Paan use and alcohol intake) was examined and HPV-DNA was found only in 9/18 (50.0%) of non-smokers.

When the use of Paan was examined, HPV-DNA was found in 9/25 (36.0%) of non-Paan users. In addition, HPV-DNA was detected in 9/24 (37.5%) of non-alcohol drinkers.

3.5.5 Site of human immunodeficiency virus oral sample

Cross tabulation for HPV-DNA frequency and other characteristics are summarised in Table 3.15. Human papillomavirus-DNA was detected in the buccal mucosa, lateral border of the tongue, attached gingiva, hard palate and anterior two thirds of tongue.

3.5.6 Relevant medical history

Human papillomavirus-DNA was detected in 8/20 (40.0%) of patients with no relevant medical history. In addition, HPV-DNA was found in 1/4 (25.0%) of males with candidal infection. The results show that HPV-DNA was found in 4/18 (22.2%) of individuals who received no corticosteroid therapy compared with 5/7 (71.4%) of those who received systemic corticosteroids. HPV was found in 9/24 (37.5%) of patients with systemic immune disease. When further stratified according to gender and immune status, HPV-DNA was detected in 1/3 (33.3%) of females compared with 8/21 (38.1%) of males. No HPV-DNA was detected in patients who had

received triple therapy. In addition, HPV-DNA was detected in 9/19 (47.4%) of patients who had received no other therapy.

3.5.7 HPV infection in relation to HIV-patients

The results for HPV infection in relation to HIV are summarised in Table 3.16. In the sample of people, none of the normal controls was HPV+ve. However, some of the HIV patients were HPV+ve. Thus the HIV patients were highly significantly more likely than normal controls to be HPV+ve in this sample ($P<0.001$, Fisher's exact test). However, since none of the controls were HPV+ve, it was not possible to calculate an odds ratio. Therefore, it was also not possible to control for the effects of any other risk factors in this analysis.

Table 3.17 Demographic details of UK normal patients

Characteristic	-ve No. (%)	-ve Female No. (%)	-ve Male No. (%)
Gender			
Female	29/50 (58.0)		
Male	21/50 (42.0)		
Age (years)			
<20	6/50(12.0)	5/29(17.2)	1/21(4.8)
20-30	11/50(22.0)	6/29(20.7)	5/21(23.8)
30-40	8/50(16.0)	4/29(13.8)	4/21(19.0)
40-50	6/50(12.0)	4/29(13.8)	2/21(9.5)
50-60	8/50(16.0)	3/29(10.3)	5/21(23.8)
60-70	4/50(8.0)	4/29(13.8)	-
70-80	4/50(8.0)	2/29(6.9)	2/21(9.5)
>80	3/50(6.0)	1/29(3.4)	2/21(9.5)
Ethnicity			
Caucasian	37/50(74.0)	23/29(79.3)	14/21(66.7)
SEA*	10/50(20.0)	5/29(17.2)	5/21(23.8)
Arab	2/50(4.0)	-	2/21(9.5)
African	1/50(2.0)	1/29(3.4)	-
Tobacco			
None	49/50(98.0)	29/29(100.0)	20/21(95.2)
Ex-smoker	1/50(2.0)	-	1/21(4.8)
Smoking			
None	49/50(98.0)	29/29(100.0)	20/21(95.2)
10-15/day	1/50(2.0)		1/21(4.8)
Paan			
None	48/50(96.0)	29/29(100.0)	19/21(90.5)
Ex-user	2/50(4.0)		2/21(9.5)
Alcohol			
None	46/50(92.0)	26/29(89.7)	20/21(95.2)
5-20u/w	4/50(8.0)	3/29(10.3)	1/21(4.8)
Total	**50/50(100.0)**	**29/29(100.0)**	**21/21(100.0)**

**SEA= South East Asian
N.B. All subjects were HPV-ve

Table 3.18 Cross tabulation for human papillomavirus in UK normal oral mucosa, according to biopsy site, relevant medical note, corticosteroids, immune status and therapies

Characteristic	-ve No. (%)	-ve Female No. (%)	-ve Male No. (%)
Biopsy site			
Buccal mucosa	11/50(22.0)	7/29(24.1)	4/21(19.0)
Lt. of tongue*	1/50(2.0)	1/29(3.4)	-
Floor of mouth	12/50(24.0)	7/29(24.1)	5/21(23.8)
Attached gingiva	1/50(2.0)	-	1/21(4.8)
Soft palate	1/50(2.0)	1/29(3.4)	-
Hard palate	4/50(8.0)	3/29(10.3)	1/21(4.8)
Ant. 2/3 of tongue**	1/50(2.0)	-	1/21(4.8)
Lip	17/50(34.0)	10/29(34.5)	7/21(33.3)
Retromolar	2/50(4.0)	-	2/21(9.5)
Relevant medical note			
None	47/50(94.0)	27/29(93.1)	20/21(95.2)
HRT***	1/50(2.0)	1/29(3.4)	-
Asthma	1/50(2.0)	-	1/21(4.8)
Penicillin allergy	1/50(2.0)	1/29(3.4)	-
Corticosteroids			
None	49/50(98.0)	28/29(96.6)	21/21(100.0)
Systemic	1/50(2.0)	1/29(3.4)	-
Immune status			
None	50/50(100.0)	29/29(100.0)	21/21(100.0)
Therapies			
None	50/50(100.0)	29/29(100.0))	21/21(100.0)
Total	**50/50(100.0)**	**29/29(100.0)**	**21/21(100.0)**

* Lt. of tongue= lateral border of the tongue
** Ant. 2/3 of tongue= anterior 2/3 of tongue
*** HRT: Hormone replacement therapy
N.B. All subjects were HPV -ve

3.6 Human papillomavirus in normal control oral mucosa-UK

The frequency of human papillomavirus DNA among oral lesions is summarised in Table 3.1. There was no HPV+ve detected in normal control oral mucosa samples. Demographic characteristics and risk factors of normal control oral mucosa samples are summarised in Table 3.17 and 3.18.

Table 3.19 Detection of human papillomavirus versus demographics and risk factors for oral squamous cell carcinoma of KSA patients with oral squamous cell carcinoma

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Gender						
Female	2/7(28.6)	5/7(71.4)				
Male	11/23(437.8)	12/23(52.2)				
Age (years)						
30-40	1/2(50.0)	1/2(50.0)	-	-	1/2(50.0)	1/2(50.0)
40-50	2/2(100.0	0/2(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)
50-60	4/7(57.1)	3/7(42.9)	0/1(0.0)	1/1(100.0)	4/6(66.7)	2/6(33.3)
60-70	2/11(18.2)	9/11(81.8)	0/2(0.0)	2/2(100.0)	2/9(22.2)	7/9(77.8)
70-80	1/3(33.3)	2/3(66.7)	-	-	1/3(33.3)	2/3(66.7)
>80	3/5(60.0)	2/5(40.0)	1/3(33.3)	2/3(66.7)	2/2(100.0)	0/2(0.0)
Ethnicity						
Caucasian	0/2(0.0)	2/2(100.0)	-	-	0/2(0.0)	2/2(100.0)
Arab	13/28(46.4)	15/28(53.6)	2/7(28.6)	5/7(71.4)	11/21(52.4)	10/21(47.6)
Tobacco						
None	13/29(44.8)	16/29(55.2)	2/7(28.6)	5/7(71.4)	11/22(50.0)	11/22(50.0)
Current	0/1(0.0)	1/1(100.0)		-	0/1(0.0)	1/1(100.0)
Smoking						
None	13/29(44.8)	16/29(55.2)	2/7(28.6)	5/7(71.4)	11/22(50.0)	11/22(50.0)
10-15/day	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Paan						
None	13/30(43.3)	17/30(56.7)	2/7(28.6)	5/7(71.4)	11/23(47.8)	12/23(52.2)
Alcohol						
None	13/30(43.3)	17/30(56.7)	2/7(28.6)	5/7(71.4)	11/23(47.8)	12/23(52.2)
Total	**13/30(43.3)**	**17/30(56.7)**	**2/7(28.6)**	**5/7(71.4)**	**11/23(47.8)**	**12/23(52.2)**

Table 3.20 Cross tabulation for human papillomavirus in KSA- oral squamous cell carcinoma samples according to biopsy site, relevant medical note, corticosteroids, immune status, and therapies

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Biopsy site						
Buccal mucosa	2/6(33.3)	4/6(66.7)	1/2(50.0)	1/2(50.0)	1/4(25.0)	3/4(75.0)
Lt. of tongue*	3/7(42.9)	4/7(57.1)	0/1(0.0)	1/1(100.0)	3/6(50.0)	3/6(50.0)
Floor of mouth	2/2(100.0)	0/2(0.0)	-	-	2/2(100.0)	0/2(0.0)
Ant. 2/3 of tongue**	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Lip	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Retromolar	4/8(50.0)	4/8(50.0)	0/1(0.0)	1/1(100.0)	4/7(57.1)	3/7(42.9)
Other ***	1/5(20.0)	4/5(80.0)	0/2(0.0)	2/2(100.0)	1/3(33.3)	2/3(66.7)
Relevant medical note						
None	12/25(48.0)	13/25(52.0)	2/5(40.0)	3/5(60.0)	10/20(50.0)	10/20(50.0)
SCC¶	0/2(0.0)	2/2(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)
Diabetes mellitus	1/2(50.0)	1/2(50.0)	0/1(0.0)	1/1(100.0)	1/1(100.0)	0/1(0.0)
Penicillin allergy	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Corticosteroids						
None	13/30(43.3)	17/30(56.7)	2/7(28.6)	5/7(71.4)	11/23(47.8)	12/23(52.2)
Immune status						
None	13/30(43.3)	17/30(56.7)	2/7(28.6)	5/7(71.4)	11/23(47.8)	12/23(52.2)
Therapies						
None	13/30(43.3)	17/30(56.7)	2/7(28.6)	5/7(71.4)	11/23(47.8)	12/23(52.2)
Total	**13/30(43.3)**	**17/30(56.7)**	**2/7(28.6)**	**5/7(71.4)**	**11/23(47.8)**	**12/23(52.2)**

* Lt. of tongue= lateral border of the tongue
** Ant. 2/3 of tongue= anterior 2/3 of tongue
*** Other sites= Data not available
¶SCC= Second primary Squamous Cell Carcinoma

Table 3.21 Human papillomavirus infection in relation to KSA-oral squamous cell carcinoma

KSA	Gender			OSCC -	OSCC +	Odds Ratio (95% CI)	P-value*
OSCC							
	ALL						
		HPV	-	50	13	N/C	<0.001
			+	0	17		
	F						
		HPV	-	29	2	N/C	<0.001
			+	0	5		
	M						
		HPV	-	21	11	N/C	<0.001
			+	0	12		

.N/C: could not be calculated because none of the normal controls were HPV +ve

* Calculated from Fisher's exact test

3.7 Human papillomavirus in oral squamous cell carcinoma - KSA

The frequency of human papillomavirus DNA among oral lesions is summarised in Table 3.1. An overall HPV-DNA positive proportion of 17/30 (56.7%) was detected in OSCC-KSA patients. Demographic characteristics and risk factors for OSCC in the KSA patients are summarised in Table 3.19

3.7.1 Gender

Human papillomavirus-DNA was detected in 5/7 (71.4%) of females compared with 12/23 (52.2%) males.

3.7.2 Age groups

When HPV-DNA was examined in different age groups, HPV infection showed a peak in the 60-70 year age group. The rate of HPV-DNA was 1/2 (50.0%) in the 30-40 year age group, and 3/7 (42.9%) in the 50-60 year age group, reaching a peak of 9/11 (81.8%) in the 60-70 year age group. The proportion falls to 2/3 (66.7%) in the 70-80 year age group and to 2/5 (40.0%) in those over 80 years of age. When stratified according to gender and age, 7/9 (77.8%) of the men aged 60-70 years were HPV infected, while 2/2 (100.0%) of the females in this age range were infected with HPV.

3.7.3 Ethnic origins

The results show that HPV-DNA was detected in both Caucasians in the samples and in 15/28 (53.6%) of Arabs. When stratified according to gender and ethnicity, HPV-DNA was detected in 5/7 (71.4%) of Arab females. However, both of the Caucasian males were HPV infected compared with 10/21 (47.6%) of the Arab males.

3.7.4 Associations with tobacco, alcohol and Paan

The association between HPV and other major risk factors for OSCC (e.g. tobacco smoking, frequency of smoking, Paan use and alcohol intake) was examined. Human papillomavirus-DNA was detected in 16/29 (55.2%) of non-smokers compared with 1/1 (100.0%) of individuals smoking 10-15/day. When stratified according to gender and smoking, none of the females in this group were current smokers, HPV-DNA was found in 11/22 (50.0%) of male non-smokers. No HPV positive samples were observed in Paan-users or alcohol drinkers of either gender.

3.7.5 Site of squamous cell carcinoma sample

Cross tabulation for HPV-DNA frequency and other characteristics are summarised in Table 3.20. Although only small numbers were present in the samples, HPV-DNA was detected in the buccal mucosa, lateral border of the tongue, lip and retromolar area. In addition, HPV-DNA was detected in 4/5 (80.0%) of patients where no data was available regarding the biopsy site.

3.7.6 Relevant medical history

Human papillomavirus-DNA was detected in 13/25 (52.0%) of patients with no relevant medical history and in 2/2 (100.0%) of patients with second-primary OSCC. In addition, HPV-DNA was found in 1/1 (100.0%) diabetic female individual and in 1/1 (100.0%) male allergic to penicillin. The results show that none of the individuals infected with HPV-DNA had received any corticosteroid therapy or had any systemic immune disease. In addition, HPV-DNA was found in 17/30 (56.7%) of patients who had received no therapy.

3.7.7 HPV infection in relation to KSA-OSCC

The results for HPV infection in relation to OSCC are summarised in Table 3.21. In the sample of people, none of the normal controls was HPV+ve. However, some of the OSCC patients were HPV+ve. Thus the OSCC patients were highly significantly more likely than normal controls to be HPV+ve in this sample ($P<0.001$, Fisher's exact test). However, since none of the controls were HPV+ve, it was not possible to calculate an odds ratio. Therefore, it was also not possible to control for the effects of any other risk factors in this analysis.

Table 3.22 Detection of human papillomavirus versus demographics and risk factors for oral squamous cell carcinoma of KSA patients with oral lichen planus

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Gender						
Female	14/19(73.7)	5/19(26.3)				
Male	9/13(69.2)	4/13(30.8)				
Age (years)						
20-30	2/3(66.6)	1/3(33.3)	1/1(100.0)	0/1(0.0)	1/2(50.0)	1/2(50.0)
30-40	4/7(57.1)	3/7(42.9)	4/6(66.7)	2/6(33.3)	0/1(0.0)	1/1(100.0)
40-50	7/9(77.8)	2/9(22.2)	5/7(71.4)	2/7(28.6)	2/2(100.0)	0/2(0.0)
50-60	6/8(75.0)	2/8(25.0)	1/2(50.0)	1/2(50.0)	5/6(83.3)	1/6(16.7)
60-70	4/5(80.0)	1/5(20.0)	3/3(100.0)	0/3(0.0)	1/2(50.0)	1/2(50.0)
Ethnicity						
SEA*	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
FEA**	2/2(100.0)	0/2(0.0)	-	-	2/2(100.0)	0/2(0.0)
Arab	21/29(72.4)	8/29(27.9)	14/19(73.7)	5/19(26.3)	7/10(70.0)	3/10(30.0)
Tobacco						
None	23/32(71.9)	9/32(28.1)	14/19(73.7)	5/19(26.3)	9/13(69.2)	4/13(30.8)
Smoking						
None	23/32(71.9)	9/32(28.1)	14/19(73.7)	5/19(26.3)	9/13(69.2)	4/13(30.8)
Paan						
None	23/32(71.9)	9/32(28.1)	14/19(73.7)	5/19(26.3)	9/13(69.2)	4/13(30.8)
Alcohol						
None	23/32(71.9)	9/32(28.1)	14/19(73.7)	5/19(26.3)	9/13(69.2)	4/13(30.8)
Total	**23/32(71.9)**	**9/32(28.1)**	**14/19(73.7)**	**5/19(26.3)**	**9/13(69.2)**	**4/13(30.8)**

*SEA=South East Asian
** FEA= Far East Asian

Table 3.23 Cross tabulation for human papillomavirus in KSA-oral lichen planus samples according to biopsy site, relevant medical note, corticosteroids, immune status and therapies

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Biopsy site						
Buccal mucosa	20/27(74.1)	7/27(25.9)	13/17(76.5)	4/17(23.5)	7/10(70.0)	3/10(30.0)
Lt. of tongue*	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Attached gingiva	0/1(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	-	-
Ant. 2/3 of tongue**	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Lip	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Other ***	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Relevant medical note						
None	14/20(70.0)	6/20(30.0)	8/12(66.7)	4/12(33.3)	6/8(75.0)	2/8(25.0)
Cutaneous disease	4/6(66.7)	2/6(33.3)	2/3(66.7)	1/3(33.3)	2/3(66.7)	1/3(33.3)
Diabetes mellitus	4/5(80.0)	1/5(20.0)	3/3(100.0)	0/3(0.0)	1/2(50.0)	1/2(50.0)
Asthma	1/1(100.0)	0/1(0.0)	1/1(100.0)	01/(0.0)	-	-
Corticosteroids						
None	23/32(71.9)	9/32(28.1)	14/19(73.7)	5/19(26.3)	9/13(69.2)	4/13(30.8)
Immune status						
None	23/32(71.9)	9/32(28.1)	14/19(73.7)	5/19(26.3)	9/13(69.2)	4/13(30.8)
Therapies						
None	23/32(71.9)	9/32(28.1)	14/19(73.7)	5/19(26.3)	9/13(69.2)	4/13(30.8)
Total	**23/32(71.9)**	**9/32(28.1)**	**14/19(73.7)**	**5/19(26.3)**	**9/13(69.2)**	**4/13(30.8)**

* Lt. of tongue= lateral border of the tongue
** Ant. 2/3 of tongue= anterior 2/3 of tongue
*** Other sites= Data not available

Table 3.24 Human papillomavirus infection in relation to KSA-oral lichen planus samples

KSA	Gender			OLP -	OLP +	Odds Ratio (95% CI)	P-value*
OLP							
	ALL	HPV	-	50	23	N/C	<0.001
			+	0	9		
	F	HPV	-	29	14	N/C	<0.001
			+	0	5		
	M	HPV	-	21	9	N/C	<0.001
			+	0	4		

N/C: could not be calculated because none of the normal controls were HPV +ve
* Calculated from Fisher's exact test

3.8 Human papillomavirus in oral lichen planus - KSA

The frequency of human papillomavirus DNA among oral lesions is summarised in Table 3.1. An overall HPV-DNA positive proportion of 9/32 (28.1%) was detected in OLP-KSA patients. Demographic characteristics and risk factors for OLP in the KSA pateints are summarised in Table 3.22

3.8.1 Gender

Human papillomavirus-DNA was detected in 5/19 (26.3%) of females compared with 4/13 (30.8%) of males.

3.8.2 Age groups

When HPV-DNA was examined in different age groups, the proportion was 1/3 (33.3%) in the 20-30 year age group, 3/7 (42.9%) in the 30-40 year age group, 2/9 (22.2%) in the 40-50 year age group and 2/8 (25.0%) in the 50-60 year age group. In addition, HPV-DNA was found in 1/5 (20.0%) in the 60-70 year age group. When stratified according to gender and age, HPV-DNA was found in 2/6 (33.3%) of the female group aged 30-40 years compared with 1/1 (100.0%) of the male group within the same age group. Human papillomavirus-DNA was detected in 1/2 (50.0%) of the female group aged 50-60 years compared with 1/6 (16.7%) of the male within the same age group.

3.8.3 Ethnic origins

Human papillomavirus-DNA was detected in 8/29 (27.9%) of Arabs compared with 1/1 (100.0%) of South East Asians. When stratified according to gender and ethnicity, HPV-DNA was found in 5/19 (26.3%) of Arab females compared with 3/10 (30.0%) of males within the same ethnic group.

3.8.4 Associations with tobacco, alcohol and Paan

The association between HPV and other major risk factors for OLP (e.g. tobacco smoking, frequency of smoking, Paan use and alcohol intake) was examined. Human papillomavirus-DNA was found in 9/32 (28.1%) of non-smokers, non-Paan users and non-alcohol drinkers.

3.8.5 Site of oral lichen planus samples

Cross tabulation for HPV-DNA frequency and other characteristics are summarised in Table 3.23. Human papillomavirus-DNA was found mostley in the buccal mucosa, and to a lesser extent, in attached gingiva and anterior two thirds of the tongue. When further stratified according to gender and biopsy site, HPV-DNA was found in 4/17 (23.5%) of females compared with 3/10 (30.0%) of males in the buccal mucosa site.

3.8.6 Relevant medical history

Human papillomavirus-DNA was detected in 6/20 (30.0%) of patients with no relevant medical history, in 2/6 (33.3%) of patients with a skin lesion and in 1/2 (50.0%) of male diabetic patients. The results show that none of the individuals infected with HPV-DNA had received any corticosteroid therapy or had any systemic immune disease. HPV-DNA was found in 9/32 (28.1%) of patients who had received no therapy.

3.8.7 HPV infection in relation to KSA-OLP

The results for HPV infection in relation to OLP are summarised in Table 3.24. In the sample of people, none of the normal controls were HPV+ve. However, some of the OLP patients were HPV+ve. Thus the OLP patients were highly significantly more likely than normal controls to be

HPV+ve in this sample (P<0.001, Fisher's exact test). Since none of the controls were HPV+ve, it was not possible to calculate an odds ratio. Therefore, it was also not possible to control for the effects of any other risk factors in this analysis.

Table 3.25 Detection of human papillomavirus versus demographics and risk factors for oral squamous cell carcinoma of KSA patients with salivary gland lesions

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Gender						
Female	5/7(71.4)	2/7(28.6)				
Male	10/13(76.9)	3/13(23.1)				
Age (years)						
20-30	2/3(66.7)	1/3(33.3)	1/1(100.0)	0/1(0.0)	1/2(50.0)	1/2(50.0)
30-40	3/5(60.0)	2/5(40.0)	0/1(0.0)	1/1(100.0)	3/4(75.0)	1/4(25.0)
40-50	5/5(100.0)	0/5(0.0)	3/3(100.0)	0/3(0.0)	2/2(100.0)	0/2(0.0)
50-60	3/4(75.0)	1/4(25.0)	0/1(0.0)	1/1(100.0)	3/3(100.0)	0/3(0.0)
60-70	2/3(66.7)	1/3(33.3)	1/1(100.0)	0/1(0.0)	1/2(50.0)	1/2(50.0)
Ethnicity						
SEA*	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
FEA**	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Arab	13/18(72.2)	5/18(27.8)	5/7(71.4)	2/7(28.6)	8/11(72.7)	3/11(27.3)
Tobacco						
None	15/20(75.0)	5/20(25.0)	5/7(71.4)	2/7(28.6)	10/13(76.9)	3/13(23.1)
Smoking						
None	15/20(75.0)	5/20(25.0)	5/7(71.4)	2/7(28.6)	10/13(76.9)	3/13(23.1)
Paan						
None	15/20(75.0)	5/20(25.0)	5/7(71.4)	2/7(28.6)	10/13(76.9)	3/13(23.1)
Alcohol						
None	15/20(75.0)	5/20(25.0)	5/7(71.4)	2/7(28.6)	10/13(76.9)	3/13(23.1)
Total	**15/20(75.0)**	**5/20(25.0)**	**5/7(71.4)**	**2/7(28.6)**	**10/13(76.9)**	**3/13(23.1)**

*SEA=South East Asian
** FEA= Far East Asian

Table 3.26 Cross tabulation for human papillomavirus in KSA-salivary gland samples according to biopsy site, relevant medical note, corticosteroids, immune status, and therapies

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Biopsy site						
Buccal mucosa	4/5(80.0)	1/5(20.0)	1/1(100.0)	0/1(0.0)	3/4(75.0)	1/4(25.0)
Floor of mouth	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Hard palate	5/9(55.6)	4/9(44.4)	1/3(33.3)	2/3(66.7)	4/6(66.7)	2/6(33.3)
Lip	1/1(100.0)	0/1(0.0)	1/1(100.01)	0/1(0.0)	-	-
Retromolar	2/2(100.0)	0/2(0.0)	1/1(100.01)	0/1(0.0)	1/1(100.0)	0/1(0.0)
Other*	2/2(100.0)	0/2(0.0)	1/1(100.01)	0/1(0.0)	1/1(100.0)	0/1(0.0)
Relevant medical note						
None	15/20(75.0)	5/20(25.0)	5/7(71.4)	2/7(28.6)	10/13(76.9)	3/13(23.1)
Corticosteroids						
None	15/20(75.0)	5/20(25.0)	5/7(71.4)	2/7(28.6)	10/13(76.9)	3/13(23.1)
Immune status						
None	15/20(75.0)	5/20(25.0)	5/7(71.4)	2/7(28.6)	10/13(76.9)	3/13(23.1)
Therapies						
None	15/20(75.0)	5/20(25.0)	5/7(71.4)	2/7(28.6)	10/13(76.9)	3/13(23.1)
Total	**15/20(75.0)**	**5/20(25.0)**	**5/7(71.4)**	**2/7(28.6)**	**10/13(76.9)**	**3/13(23.1)**

* Other sites= Data not available

Table 3.27 Human papillomavirus infection in relation to KSA salivary gland samples

KSA	Gender				Odds Ratio (95% CI)	P-value
Salivary						
	ALL		**Salivary**			
			-	+		
		HPV -	50	15	N/C	<0.001
		HPV +	0	5		
	F		**Salivary**			
			-	+		
		HPV -	29	5	N/C	<0.001
		HPV +	0	2		
	M		**Salivary**			
			-	+		
		HPV -	21	10	N/C	<0.001
		HPV +	0	3		

N/C: could not be calculated because none of the normal controls were HPV +ve
* Calculated from Fisher's exact test

3.9 Human papillomavirus in salivary gland samples from KSA

The frequency of human papillomavirus DNA among oral lesions is summarised in Table 3.1. An overall HPV-DNA positive proportion of 5/25 (25.0%) was detected in salivary gland lesions-KSA patients. Demographic characteristics and risk factors for salivary gland lesions in the KSA pateints are summarised in Table 3.25

3.9.1 Gender

Human papillomavirus-DNA was detected in 2/7 (28.6%) of females compared with 3/13 (23.1%) of males.

3.9.2 Age groups

When HPV-DNA was examined in different age groups, HPV-DNA was 1/3 (33.3%) in patients in the 20-30 year age group, 1/4 (25.0%) in the 30-40 year age group and 1/3 (33.3%) in the 60-70 year age group. However, HPV was positive in 1/1 (100.0%) female aged 30-40 years compared with 1/4 (25.0%) of males within the same age group.

3.9.3 Ethnic origins

Human papillomavirus-DNA was detected in 5/18 (27.8%) of Arabs. When stratified according to gender and ethnicity, HPV-DNA was found in 2/7 (28.6%) of female Arab descendents compared with 3/11 (27.3%) of males individuals within the same ethnic group.

3.9.4 Association with tobacco, alcohol and Paan

The association between HPV and other major risk factors for salivary gland lesions (e.g. tobacco smoking, frequency of smoking, Paan use and

alcohol intake) was examined. Human papillomavirus-DNA was found in 5/20 (25.0%) of non-smokers, non-Paan users and non-alcohol drinkers.

3.9.5 Site of salivary gland samples

Cross tabulation for HPV-DNA frequency and other characteristics are summarised in Table 3.26. Human papillomavirus-DNA was found mostly in the hard palate and, to a lesser extent, in the buccal mucosa. When further stratified according to gender and biopsy site, HPV-DNA was found in 2/3 (66.7%) of the hard palate of females compared with 2/6 (33.3%) of males.

3.9.6 Relevant medical history

The results show that none of the individuals infected with HPV-DNA had received any corticosteroid therapy or had any systemic immune disease. In addition, HPV-DNA was found in patients who had received no therapy.

3.9.7 HPV infection in relation to KSA-salivary lesions

The results for HPV infection in relation to salivary gland samples are summarised in Table 3.27. In the sample of people, none of the normal controls was HPV+ve. However, some of the salivary gland samples were HPV+ve. Thus the salivary gland lesions patients were highly significantly more likely than normal controls to be HPV+ve in this sample ($P<0.001$, Fisher's exact test). Since none of the controls were HPV+ve, it was not possible to calculate an odds ratio. Therefore, it was also not possible to control for the effects of any other risk factors in this analysis.

Table 3.28 The relative risk (OR) of human papillomavirus infection in oral epithelial dysplasia compared to oral squamous cell carcinoma

UK	Gender			OED	OSCC	Odds Ratio (95 % CI)	P-value
	ALL						
		HPV	-	26	7	5.278 (1.90, 14.64)	<0.001
			+	19	27		
	F						
		HPV	-	12	2	6.00 (1.059, 34.0)	0.043
			+	10	10		
	M						
		HPV	-	14	5	5.289 (1.44, 19.45)	0.012
			+	9	17		

3.10 Relative risk of HPV in OED compared to OSCC

Table 3.28 compares the viral prevalence of HPV in OED and OSCC. The OR results indicate that OSCC patients are approximately 5.3 times more likely to be infected with HPV than OED patients (95% CI = 1.9, 14.6). When stratified according to gender, the results were essentially unchanged.

Table 3.29 Single and double infection of HPV and HHV-8 in oral squamous cell carcinoma

	Non-infected No. (%)	Single HPV Infection No. (%)	Single HHV-8 Infection No. (%)	Double infections No. (%)	Total No. (%)
UK-OSCC	7(20.6)	24(70.6)	0(0.0)	3(8.8)	34(100.0)
KSA-OSCC	12(40.0)	11(36.7)	1(3.3)	6(20.0)	30(100.0)
All-OSCC	19(29.7)	35(54.7)	1(1.6)	9(14.0)	64(100.0)

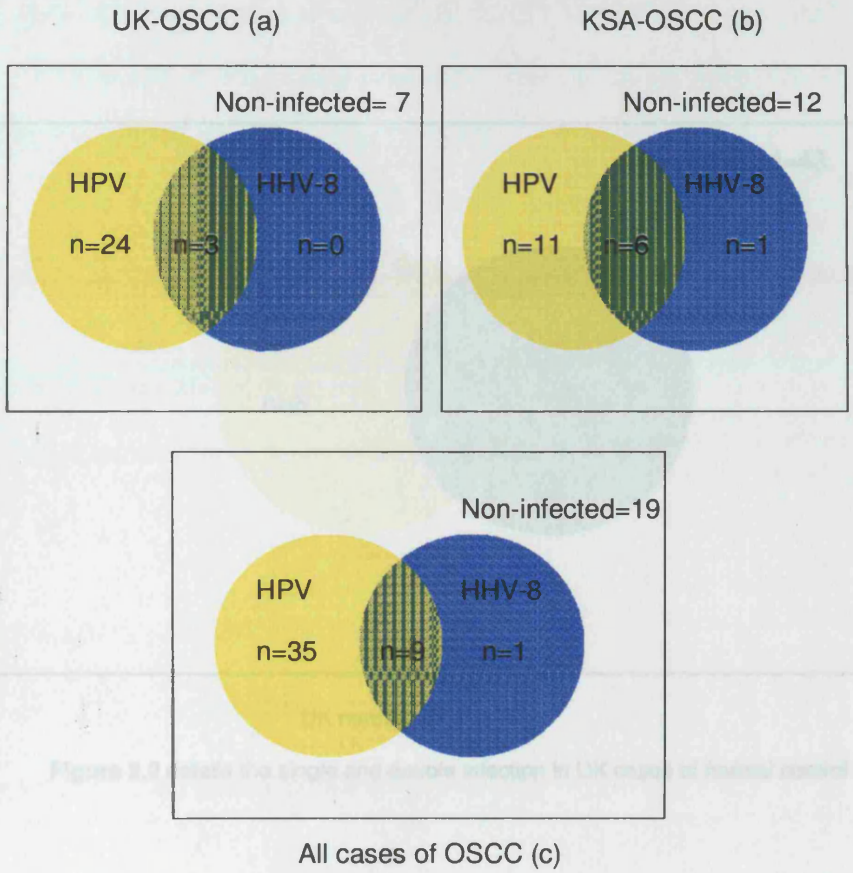
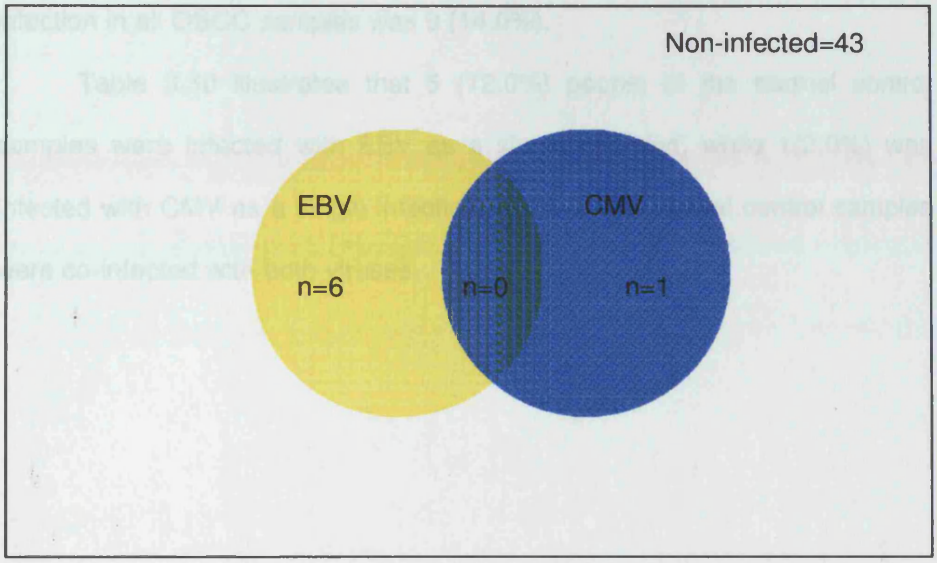


Figure 3.1

(a) details the single and double infections in UK-OSCC
(b) details the single and double infections in KSA-OSCC
(c) details the single and double infections in ALL cases of OSCC

Table 3.30 Single and double infection of EBV and CMV in normal control samples from the UK

	Non-infected No. (%)	Single EBV infection No. (%)	Single CMV infection No. (%)	Double infections No. (%)	Total No. (%)
Normal control	43(86.0)	6(12.0)	1(2.0)	0(0.0)	50(100.0)



UK normal controls

Figure 3.2 details the single and double infection in UK cases of normal control

3.11 Single and double infections

Table 3.29 shows that HPV was present as a single infection in 24 (70.6%) of UK-OSCC samples, 11 (36.7%) of KSA-OSCC samples and 35 (54.7%) in all OSCC samples, while HHV-8 was present as a single infection in 0 (0.0%) of UK-OSCC samples, 1(3.3%) of KSA-OSCC samples and in 1(1.6%) of all OSCC samples. However, 3 people (8.8%) were co-infected with both HPV and HHV-8 viruses of UK-OSCC. Similarly, 6 people (20.0%) were co-infected in KSA-OSCC samples. The combined presence of co-infection in all OSCC samples was 9 (14.0%).

Table 3.30 illustrates that 6 (12.0%) people of the normal control samples were infected with EBV as a single infection, while 1(2.0%) was infected with CMV as a single infection. None of the normal control samples were co-infected with both viruses.

Chapter 4

Human herpesvirus- 8 Results

Table 4.1 Frequency of human herpesvirus-8 in examined oral mucosal and salivary gland lesions

Country	Lesion	Gender	-ve No. (%)	+ve No. (%)
UK				
	OSCC	All	31/34 (91.2)	3/34 (8.8)
		F	10/12 (83.3)	2/12 (16.7)
		M	21/22 (95.5)	1/22 (4.5)
	PVL	All	9/12 (75.0)	3/12 (25.0)
		F	3/6 (50.0)	3/6 (50.0)
		M	6/6 (100)	-
	OED	All	44/45 (97.8)	1/45 (2.2)
		F	22/22 (100.0)	-
		M	22/23 (95.7)	1/23 (4.3)
	OLP	All	27/30 (90.0)	3/30 (10.0)
		F	12/14 (85.7)	2/14 (14.3)
		M	15/16 (93.8)	1/16 (6.3)
	HIV	All	13/25 (52.0)	12/25 (48.0)
		F	1/3 (33.3)	2/3 (66.7)
		M	12//22 (54.5)	10/22 (45.5)
KSA				
	OSCC	All	23/30 (76.7)	7/30 (23.3)
		F	6/7 (85.7)	1/7 (14.3)
		M	17/23 (73.9)	6/23 (26.1)
	OLP	All	20/32 (62.5)	12/32 (37.5)
		F	11/19 (57.9)	8/19 (42.1)
		M	9/13 (69.2)	4/13 (30.8)
	Salivary	All	15/25 (75.0)	5/25 (25.0)
		F	5/7 (71.4)	2/7 (28.6)
		M	10/13 (76.9)	3/13 (23.1)
Normal- control				
	UK	All	50/50(100.0)	0/50(0.0)
		F	29/29(100.0)	0/21(0.0)
		M	21/21(100.0)	0/21(0.0)

OSCC = Oral squamous cell carcinoma
PVL = Proliferative verrucous leukoplakia
OED = Oral epithelial dysplasia
OLP = Oral lichen planus
HIV = Human immunodeficiency virus

Table 4.2 Detection of human herpes virus-8 versus demographics and risk factors for oral squamous cell carcinoma of UK patients with oral squamous cell carcinoma

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Gender						
Female	10/12(83.3)	2/12(16.7)				
Male	21/22(95.5)	1/22(4.5)				
Age (years)						
30-40	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
50-60	8/8(100.0)	0/8(0.0)	4/4(100.0)	0/4(0.0)	4/4(100.0)	0/4(0.0)
60-70	5/5(100.0)	0/5(0.0)	2/2(100.0)	0/2(0.0)	3/3(100.0)	0/3(0.0)
70-80	10/12(83.3)	2/12(16.7)	1/2(50.0)	1/2(50.0)	9/10(90.0)	1/10(10.0)
>80	7/8(87.5)	1/8(12.5)	3/4(75.0)	1/4(25.0)	4/4(100.0)	0/4(0.0)
Ethnicity						
Caucasian	24/26(92.3)	2/26(7.7)	5/6(83.3)	1/6(16.7)	19/20(95.0)	1/20(5.0)
SEA*	6/7(85.7)	1/7(14.3)	5/6(83.3)	1/6(16.7)	1/1(100.0)	0/1(0.0)
N/A**	1/1(110.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Tobacco						
None	28/31(90.3)	3/31(9.7)	10/12(83.3)	2/12(16.7)	18/19(94.7)	1/19(5.3)
Current	3/3(100.0)	0/3(0.0)	-	-	3/3(100.0)	0/3(0.0)
Smoking						
None	28/31(90.3)	3/31(9.7)	10/12(83.3)	2/112(16.7)	18/19(94.7)	1/19(5.3)
10-15/day	2/2(100.0)	0/2(0.0)	-	-	2/2(100.0)	0/2(0.0)
>20/day	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Paan						
None	28/30(93.3)	2/30(6.7)	9/10(90.0)	1/10(10.0)	19/20(95.0)	1/20(5.0)
Current	3/4(75.0)	1/4(25.0)	1/2(50.0)	1/2(50.0)	2/2(100.0)	0/2(0.0)
Alcohol						
None	27/30(90.0)	3/30(10.0)	10/12(83.3)	2/112(16.7)	17/18(94.4)	1/18(5.6)
5-20 u/w***	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
N/A	3/3(100.0)	0/3(0.0)	-	-	3/3(100.0)	0/3(0.0)
Total	**31/34(91.2)**	**3/34(8.8)**	**10/12(83.3)**	**2/12(16.7)**	**21/22(95.5)**	**1/22(4.5)**

*SEA=South East Asian
** N/A= Data not available
*** u/w = units per week

Table 4.3 Cross tabulation for human herpesvirus-8 in UK-oral squamous cell carcinoma samples according to biopsy site, relevant medical note, corticosteroids, immune status, and therapies

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Biopsy site						
Buccal mucosa	5/6(83.3)	1/6(16.7)	3/4(75.0)	1/4(25.0)	2/2(100.0)	0/2(0.0)
Lt. of tongue*	5/5(100.0)	0/5(0.0)	-	-	5/5(100.0)	0/5(0.0)
Floor of mouth	5/5(100.0)	0/5(0.0)	-	-	5/5(100.0)	0/5(0.0)
Soft palate	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Hard palate	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Ant. 2/3 of tongue**	1/2(50.0)	1/2(50.0)	1/2(50.0)	1/2(50.0)	-	-
Lip	5/5(100.0)	0/5(0.0)	2/2(100.0)	0/2(0.0)	3/3(100.0)	0/3(0.0)
Commisure	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Retromolar	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Other ***	7/7(100.0)	0/7(0.0)	3/3(100.0)	0/3(0.00	4/4(100.00	0/4(0.0)
Relevant medical note						
None	15/15(100.0)	0/15(0.0)	4/4(100.0)	0/4(0.0)	11/11(100.0)	0/11(0.0)
SCC¶	15/18(83.3)	3/18(16.7)	6/8(75.0)	2/8(25.0)	9/10(90.0)	1/10(10.0)
Candida	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Corticosteroids						
None	31/34(91.2)	3/34(8.8)	10/12(16.7)	2/12(16.7)	21/22(95.5)	1/22(4.5)
Immune status						
None	29/32(90.6)	3/32(9.4)	8/10(80.0)	2/10(20.0)	21/22(95.5)	1/22(4.5)
Systemic	2/2(100.0)	0/2(0.0)	2/2(100.0)	0/2(0.0)	-	-
Therapies						
None	25/26(96.2)	1/26(3.8)	8/8(100.0)	0/8(0.0)	17/18(94.4)	1/18(5.6)
Chemotherapy	5/6(83.3)	1/6(16.7)	2/3(66.7)	1/3(33.3)	3/3(100.0)	0/3(0.0)
Radiotherapy	1/2(50.0)	1/2(50.0)	0/1(0.0)	1/1(100.0)	1/1(100.0)	0/1(0.0)
Total	**31/34(91.2)**	**3/34(8.8)**	**10/12(83.3)**	**2/12(16.7)**	**21/22(95.5)**	**1/22(4.5)**

* Lt. of tongue= lateral border of the tongue
** Ant. 2/3 of tongue= anterior 2/3 of tongue
*** Other sites= Data not available
¶SCC= Second primary Squamous Cell Carcinoma

Table 4.4 Human herpesvirus-8 infection in relation to UK-oral squamous cell carcinoma samples

UK	Gender			OSCC -	OSCC +	Odds Ratio (95% CI)	P-value*
OSCC							
	ALL						
		HHV8	-	50	31	N/C	<0.001
			+	0	3		
	F						
		HHV8	-	29	10	N/C	<0.001
			+	0	2		
	M						
		HHV8	-	21	21	N/C	<0.001
			+	0	1		

N/C: could not be calculated because none of the normal controls were HHV-8 +ve

Calculated from Fisher's exact test

4.1 Human herpesvirus- 8 in oral squamous cell carcinoma - UK

The frequency of human herpesvirus-8 DNA among oral lesions is summarised in Table 4.1. An overall HHV-8 DNA positive proportion of 3/34 (8.8%) was detected in OSCC-UK patients. Demographic characteristics and risk factors for OSCC in the UK patients are summarised in Table 4.2.

4.1.1 Gender

Human herpesvirus-8 DNA was detected in 2/12 (16.7%) of females compared with 1/22 (4.5%) iof males.

4.1.2 Age groups

When HHV-8 DNA was examined in different age groups, the proportion of HHV-8 DNA was 2/12 (16.7%) of 70-80 year age group, and 1/4 (25.0%) in the > 80 years female group. HHV-8 was found in 1/2 (50.0%) of the females aged 70-80 years compared with 1/10 (10.0%) of males within the same age group.

4.1.3 Ethnic origins

Human herpesvirus-8 DNA was detected in 2/26 (7.7%) of Caucasians compared with 1/6 (16.7%) in female South East Asians. When stratified according to gender and ethnicity, HHV-8 DNA was detected in 1/6 (16.7%) of females in both ethnic groups. HHV-8 DNA was detected in 1/20 (5.0%) of the Caucasian males.

4.1.4 Association with tobacco, alcohol and Paan

The association between HHV-8 and other major risk factors for OSCC (e.g. tobacco smoking, frequency of smoking, Paan use and alcohol

intake) was examined. Human herpesvirus-8 DNA was detected only in 3/31 (9.7%) of non-smokers. When the use of Paan was examined, HHV-8 DNA was found in 2/30 (6.7%) of non-Paan users compared with 1/2 (50.0%) of female Paan users. Human herpesvirus-8 DNA was detected in only 3/30 (10.0%) of non-alcohol drinkers.

4.1.5 Site of oral squamous cell carcinoma samples

Cross tabulation for HHV-8 DNA frequency and other characteristics are summarised in Table 4.3. Only small numbers were present in the samples, HHV-8 DNA was detected in the buccal mucosa, hard palate and anterior two thirds of tongue. No difference was readily apparent as regards the biopsy site and HHV-8 infection was found in similar proportions in both genders.

4.1.6 Relevant medical history

Human herpesvirus-8 DNA was detected in 3/18 (16.7%) of patients with second-primary OSCC. When stratified according to gender and medical history, HHV-8 was detected in 2/8 (25.0%) of female patients with second-primary OSCC compared with 1/10 (10.0%) of males within the same group.

The results show that none of the individuals infected with HHV-8 DNA had received any corticosteroid therapy or had any immune diseases. In patients receiving chemotherapy or radiotherapy, HHV-8 DNA was detected only in female individuals receiving therapy. Human herpesvirus-8 DNA was found in 1/3 (33.3%) of female patients who had received chemotherapy and in 1/1 (100.0%) of female patients who had received radiotherapy. However,

HHV-8 DNA was found in 1/18 (5.6%) of male patients who had received no therapy.

4.1.7 HHV-8 infection in relation to UK-OSCC

The results for HHV-8 infection in relation to OSCC are summarised in Table 4.4. In the sample of people, none of the normal controls was HHV-8+ve. However, some of the OSCC patients were HHV-8 +ve. Thus, the OSCC patients were highly significantly more likely than normal controls to be HHV-8+ve in this sample ($P<0.001$, Fisher's exact test). Since none of the controls were HHV-8+ve, it is not possible to calculate an odds ratio. Therefore, it was also not possible to control for the effects of any of the risk factors in this analysis.

Table 4.5 Detection of human herpesvirus-8 versus demographics and risk factors for oral squamous cell carcinoma of UK pateints with proliferative verrucous leukoplakia

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Gender						
Female	3/6(50.0)	3/6(50.0)				
Male	6/6(100.0)	0/6(0.0)				
Age (years)						
40-50	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
50-60	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
60-70	1/3(33.3)	2/3(66.7)	0/2(0.0)	2/2(100.0)	1/1(100.0)	0/1(0.0)
70-80	5/6(83.3)	1/6(16.7)	2/3(66.7)	1/3(33.3)	3/3(100.0)	0/3(0.0)
>80	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Ethnicity						
Caucasian	3/5(60.0)	2/5(40.0)	2/4(50.0)	2/4(50.0)	1/1(100.0)	0/1(0.0)
N/A*	6/7(85.7)	1/7(14.3)	1/2(50.0)	1/2(50.0)	5/5(100.0)	0/5(0.0)
Tobacco						
None	7/10(70.0)	3/10(30.0)	3/6(50.0)	3/6(50.0)	4/4(100.0)	0/4(0.0)
Current	2/2(100.0)	0/2(0.0)	-	-	2/2(100.0)	0/2(0.0)
Smoking						
None	7/10(70.0)	3/10(30.0)	3/6(50.0)	3/6(50.0)	4/4(100.0)	0/4(0.0)
>20/day	2/2(100.0)	0/2(0.0)	-	-	2/2(100.0)	0/2(0.0)
Paan						
None	8/11(72.7)	3/11(27.3)	3/6(50.0)	3/6(50.0)	5/5(100.0)	0/5(0.0)
Current	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Alcohol						
None	8/11(72.7)	3/11(27.3)	3/6(50.0)	3/6(50.0)	5/5(100.0)	0/5(0.0)
>20 u/w**	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Total	**9/12(75.0)**	**3/12(25.0)**	**3/6(50.0)**	**3/6(50.0)**	**6/6(100.0)**	**0/6(0.0)**

* N/A= Data not available
** u/w = units per week

Table 4.6 Cross tabulation for human herpesvirus-8 in UK–proliferative verrucous leukoplakia samples according to biopsy site, relevant medical note, corticosteroids, immune status, and therapies

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Biopsy site						
Buccal mucosa	2/4(50.0)	2/4(50.0)	2/4(50.0)	2/4(50.0)	-	-
Lt. of tongue*	2/2(100.0)	0/2(0.0)	-	-	2/2(100.0)	0/2(0.0)
Floor of mouth	2/2(100.0)	0/2(0.0)	-	-	2/2(100.0)	0/2(0.0)
Hard palate	0/1(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	-	-
Ant. 2/3 of tongue**	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Lip	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Retromolar	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Relevant medical note						
None	2/3(66.7)	1/3(33.3)	1/2(50.0)	1/2(50.0)	1/1(100.0)	0/1(0.0)
SCC***	4/6(66.7)	2/6(33.3)	1/3(33.3)	2/3(66.7)	3/3(100.0)	0/3(0.0)
Candida	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Penicillin allergy	2/2(100.0)	0/2(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)
Corticosteroids						
None	9/12(75.0)	3/12(25.0)	3/6(50.0)	3/6(50.0)	6/6(100.0)	0/6(0.0)
Immune status						
None	9/12(75.0)	3/12(25.0)	3/6(50.0)	3/6(50.0)	6/6(100.0)	0/6(0.0)
Therapies						
None	9/11(81.8)	2/11(18.2)	3/5(60.0)	2/5(40.0)	6/6(100.0)	0/6(0.0)
Chemotherapy	0/1(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	-	-
Total	**9/12(75.0)**	**3/12(25.0)**	**3/6(50.0)**	**3/6(50.0)**	**6/6(100.0)**	**0/6(0.0)**

* Lt. of tongue= lateral border of the tongue
** Ant. 2/3 of tongue= anterior 2/3 of tongue
*** SCC= Second primary Squamous Cell Carcinoma

Table 4.7 Human herpesvirus-8 infection in relation to UK-proliferative verrucous leukoplakia samples

UK	Gender				Odds Ratio (95% CI)	P-value*
PVL						
	ALL		**PVL**			
			-	+		
		HHV8 -	50	9	N/C	<0.001
		HHV8 +	0	3		
	F		**PVL**			
			-	+		
		HHV8 -	29	3	N/C	<0.001
		HHV8 +	0	3		
	M		**PVL**			
			-	+		
		HHV8 -	21	6	N/C	<0.001
		HHV8 +	0	0		

N/C: could not be calculated because none of the normal controls were HHV-8 +ve
*Calculated from Fisher's exact test

4.2 Human herpesvirus- 8 in proliferative verrucous leukoplakia - UK

The frequency of human herpesvirus-8 DNA among oral lesions is summarised in Table 4.1. An overall HHV-8 DNA positive proportion of 3/12 (25.0%) was detected in PVL-UK patients. Demographic characteristics and risk factors for PVL in the UK pateints are summarised in Table 4.5.

4.2.1 Gender

Human herpesvirus-8 DNA was detected in 3/6 (50.0%) females only. None of the males were infected with HHV-8.

4.2.2 Age groups

When HHV-8 DNA was examined in different age groups, the proportion of HHV-8 DNA was 2/3 (66.7%) in the 60-70 year age group, and 1/6 (16.7%) in the 70-80 year age female group, and it was not detected in any other age group.

4.2.3 Ethnic origins

Human herpesvirus-8 DNA was detected in 2/5 (40.0%) of female Caucasians compared with 1/7 (14.3%) in female with no recorded ethnicity.

4.2.4 Association with tobacco, alcohol and Paan

The association between HHV-8 and other major risk factors for PVL (e.g. tobacco smoking, frequency of smoking, Paan use and alcohol intake) was examined. Human herpesvirus-8 was detected in 3/10 (30.0%) of non-smokers and no HHV-8 was detected among smokers group. When the use of Paan and alcohol drinking were examined, HHV-8 DNA was found in 3/11

(27.3%) of both non-Paan users and non-alcohol drinkers. HHV-8 was not detected in any of the Paan-users nor alcohol drinkers.

4.2.5 Site of proliferative verrucous leukoplakia samples

Cross tabulation for HHV-8 DNA frequency and other characteristics are summarised in Table 4.6. Only small numbers were present in the samples, HHV-8 DNA was detected in the buccal mucosa and hard palate.

4.2.6 Relevant medical history

Human herpesvirus-8 DNA was detected in 2/6 (33.3%) of patients with second-primary OSCC, and in 1/3 (33.3%) of patients with no relevant medical history. The results show that none of the individuals infected with HHV-8 DNA had received any corticosteroid therapy or had any immune diseases. In patients receiving chemotherapy, HHV-8 DNA was detected in one of the females 1/1(100.0%) and in 2/5 (40.0%) of patients who had received no therapy.

4.2.7 HHV-8 infection in relation to UK-PVL

The results for HHV-8 infection in relation to PVL are summarised in Table 4.7. In the sample of people, none of the normal controls was HHV-8+ve. However, some of the PVL patients were HHV-8 +ve. Thus the PVL patients were highly significantly more likely than normal controls to be HHV-8+ve in this sample ($P<0.001$, Fishers exact test). Since none of the controls were HHV-8+ve, it was not possible to calculate an odds ratio. Therefore, it was also not possible to control for the effects of any of the risk factors in this analysis.

Table 4.8 Detection of human herpesvirus-8 versus demographics and risk factors for oral squamous cell carcinoma of UK patients with oral epithelial dysplasia

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Gender						
Female	22/22(100.0)	0/22(0.0)				
Male	22/23(95.7)	1/23(4.3)				
Age (years)						
20-30	1/1(100.0)	0/1(0.0)		-	1/1(100.0)	0/1(0.0)
30-40	2/2(100.0)	0/2(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)
40-50	3/3(100.0)	0/3(0.0)	1/1(100.0)	0/1(0.0)	2/2(100.0)	0/2(0.0)
50-60	16/16(110.0)	0/16(0.0)	6/6(100.0)	0/6(0.0)	10/10(100.0)	0/10(0.0)
60-70	13/14(92.9)	1/14(7.1)	7/7(100.0)	0/7(0.0)	6/7(85.7)	1/7(14.3)
70-80	9/9((100.0)	0/9(0.0)	7/7(100.0)	0/7(0.0)	2/2(100.0)	0/2(0.0)
Ethnicity						
Caucasian	29/30(96.7)	1/30(3.3)	14/14(100.0)	0/14(0.0)	15/16(93.8)	1/16(6.3)
SEA*	6/6(100.0)	0/6(0.0)	4/4(100.0)	0/4(0.0)	2/2(100.0)	0/2(0.0)
N/A**	9/9(100.0)	0/9(0.0)	4/4(100.0)	0/4(0.0)	5/5(100.0)	0/5(0.0)
Tobacco						
None	24/24(100.0)	0/24(0.0)	15/15(100.0)	0/15(0.0)	9/9(100.0)	0/9(0.0)
Ex-smoker	2/3(66.7)	1/3(33.3)	-	-	2/3(66.7)	1/3(33.3)
Current	18/18(100.0)	0/18(0.0)	7/7(100.0)	0/7(0.0)	11/11(100.0)	0/11(0.0)
Smoking						
None	24/24(100.0)	0/24(0.0)	15/15(100.0)	0/15(0.0)	9/9(100.0)	0/9(0.0)
10-15/day	12/12(100.0)	0/12(0.0)	4/4(100.0)	0/4(0.0)	8/8(100.0)	0/8(0.0)
>20/day	8/9(88.9)	1/9(11.1)	3/3(100.0)	0/3(0.0)	5/6(83.3)	1/6(16.7)
Paan						
None	39/40(97.5)	1/40(2.5)	19/19(100.0)	0/19(0.0)	20/21(95.2)	1/21(4.8)
Current	5/5(100.0)	0/5(0.0)	3/3(100.0)	0/3(0.0)	2/2(100.0)	0/2(0.0)
Alcohol						
None	23/23(100.0)	0/23(0.0)	11/11(100.0)	0/11(0.0)	12/12(100.0)	0/12(0.0)
<5	4/4(100.0)	0/4(0.0)	3/3(100.0)	0/3(0.0)	1/1(100.0)	0/1(0.0)
5-20	3/3(100.0)	0/3(0.0)	1/1(100.0)	0/1(0.0)	2/2(100.0)	0/2(0.0)
>20 u/w***	11/12(91.7)	1/12(8.3)	6/6(100.0)	0/6(0.0)	5/6(83.3)	1/6(16.7)
N/A	3/3(100.0)	0/3(0.0)	1/1(100.0)	0/1(0.0)	2/2(100.0)	0/2(0.0)
Total	**44/45(97.8)**	**1/45(2.2)**	**22/22(100.0)**	**0/22(0.0)**	**22/23(95.7)**	**1/23(4.3)**

*SEA=South East Asian
**N/A= Data not available
*** u/w = units per week

Table 4.9 Cross tabulation for human herpesvirus-8 in UK-oral epithelial dysplasia samples according to biopsy site, relevant medical note, corticosteroids, immune status, and therapies

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Biopsy site						
Buccal mucosa	8/8(100.0)	0/8(0.0)	3/3(100.0)	0/3(0.0)	5/5(100.0)	0/5(0.0)
Lt. of tongue*	12/12(100.0)	0/12(0.0)	6/6(100.0)	0/6(0.0)	6/6(100.0)	0/6(0.0)
Floor of mouth	3/3(100.0)	0/3(0.0)	2/2(100.0)	0/2(0.0)	1/1(100.0)	0/1(0.0)
Attached gingiva	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Soft palate	2/2(100.0)	0/2(0.0)	-	-	2/2(100.0)	0/2(0.0)
Hard palate	1/2(50.0)	1/2(50.0)	-	-	1/2(50.0)	1/2(50.0)
Ant. 2/3 of tongue**	4/4(100.0)	0/4(0.0)	4/4(100.0)	0/4(0.0)	-	-
Lip	2/2(100.0)	0/2(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)
Commisure	9/9(100.0)	0/9(0.0)	3/3(100.0)	0/3(0.0)	6/6(100.0)	0/6(0.0)
Retromolar	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Salivary	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Relevant medical note						
None	20/20(100.0)	0/20(0.0)	11/11(100.0)	0/11(0.0)	9/9(100.0)	0/9(0.0)
SCC¶	7/7(100.0)	0/7(0.0)	2/2(100.0)	0/2(0.0)	5/5(100.0)	0/5(0.0)
HRT***	5/5(100.0)	0/5(0.0)	5/5(100.0)	0/5(0.0)	-	-
Asthma	2/2(100.0)	0/2(0.0)	-	-	2/2(100.0)	0/2(0.0)
Submucous fibrosis	4/4(100.0)	0/4(0.0)	2/2(100.0)	0/2(0.0)	2/2(100.0)	0/2(0.0)
Candida	4/5(80.0)	1/5(20.0)	2/2(100.0)	0/2(0.0)	2/3(66.7)	1/3(33.3)
Diabetes mellitus	1/1(0.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Arthritis	1/1(0.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Corticosteroids						
None	44/45(97.8)	1/45(2.2)	22/22(100.0)	0/22(0.0)	22/23(95.7)	1/23(4.3)
Immune status						
None	42/43(97.7)	1/43(2.3)	21/21(100.0)	0/21(0.0)	21/22(95.5)	1/22(4.5)
Systemic	2/2(100.0)	0/2(0.0)	1/1(100.0)	0/1(0.0)	1/1(0.0)	0/1(0.0)
Therapies						
None	40/41(97.6)	1/41(2.4)	18/18(100.0)	0/18(0.0)	22/23(95.7)	1/23(4.3)
Chemotherapy	4/4(100.0)	0/4(0.0)	4/4(100.0)	0/4(0.0)	-	-
Total	**44/45(97.8)**	**1/45(2.2)**	**22/22(100.0)**	**0/22(0.0)**	**22/23(95.7)**	**1/23(4.3)**

* Lt. of tongue= lateral border of the tongue
** Ant. 2/3 of tongue= anterior 2/3 of tongue
***HRT=Hormone replacement therapy
¶SCC= second primary Squamous Cell Carcinoma

Table 4.10 Human herpesvirus-8 infection in relation to UK-oral epithelial dysplasia samples

UK	Gender				Odds Ratio (95% CI)	P-value*
OED						
	ALL		**OED**			
			-	+		
		HHV8 -	50	44	N/C	<0.001
		HHV8 +	0	1		
	F		**OED**			
			-	+		
		HHV8 -	29	22	N/C	<0.001
		HHV8 +	0	0		
	M		**OED**			
			-	+		
		HHV8 -	21	22	N/C	<0.001
		HHV8 +	0	1		

N/C: could not be calculated because none of the normal controls were HHV-8 +ve
* Calculated from Fisher's exact test

4.3 Human herpesvirus- 8 in oral epithelial dysplasia - UK

The frequency of human herpesvirus-8 DNA among oral lesions is summarised in Table 4.1. An overall HHV-8 DNA positive proportion of 1/45 (2.2%) was detected in OED-UK patients. Demographic characteristics and risk factors for OED in the UK pateints are summarised in Table 4.8.

4.3.1 Human herpesvirus-8 in OED demographic Characteristics

Human herpesvirus-8 was only detected in one sample. This person was a Caucasian male aged 60-70 who was an ex-smoker who drinks >20 units of alcohol per week. He had no relevant medical history and the site of the lesion was the hard palate. Since only one sample had detectable HHV-8 it was not possible to calculate ORs for infection and logistic regression was not undertaken.

Table 4.11 Detection of human herpesvirus-8 versus demographics and risk factors for oral squamous cell carcinoma of UK patients with oral lichen planus

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Gender						
Female	12/14(85.7)	2/14(14.3)				
Male	15/16(93.8)	1/16(6.3)				
Age (years)						
40-50	6/7(85.7)	1/7(14.3)	3/4(75.0)	1/4(25.0)	3/3(100.0)	0/3(0.0)
50-60	9/10(90.0)	1/10(10.0)	4/5(80.0)	1/5(20.0)	5/5(100.0)	0/5(0.0)
60-70	8/8(100.0)	0/8(0.0)	3/3(100.0)	0/3(0.0)	5/5(100.0)	0/5(0.0)
70-80	4/5(80.0)	1/5(20.0)	2/2(100.0)	0/2(0.0)	2/3(66.7)	1/3(33.3)
Ethnicity						
Caucasian	15/16(93.8)	1/16(6.3)	7/8(87.5)	1/8(12.5)	8/8(100.0)	0/8(0.0)
SEA*	4/5(80.0)	1/5(20.0)	2/3(66.7)	1/3(33.3)	2/2(100.0)	0/2(0.0)
N/A**	8/9(88.9)	1/9(11.1)	3/3(100.0)	0/3(0.0)	5/6(83.3)	1/6(16.7)
Tobacco						
None	23/26(88.5)	3/26(11.5)	11/13(84.6)	2/13(15.4)	12/13(92.3)	1/13(7.7)
Ex-smoker	1/1(100.0)	0/1(0.0)	-	-	2/2(100.0)	0/2(0.0)
Current	3/3(100.0)	0/3(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)
Smoking						
None	23/26(88.5)	3/26(11.5)	11/13(84.6)	2/13(15.4)	12/13(92.3)	1/13(7.7)
<5 /day	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
10-15 /day	2/2(100.0)	0/2(0.0)	-	-	2/2(100.0)	0/2(0.0)
>20 /day	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Paan						
None	26/29(89.7)	3/29(10.3)	11/13(84.6)	2/13(15.4)	15/16(93.7)	1/16(6.3)
Current	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Alcohol						
None	20/23(87.0)	3/23(13.0)	10/12(83.3)	2/12(16.7)	10/11(91.0)	1/11(9.0)
5-20 u/w***	2/2(100.0)	0/2(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)
>20 u/w	2/2(100.0)	0/2(0.0)	-	-	2/2(100.0)	0/2(0.0)
N/A	3/3(100.0)	0/3(0.0)	1/1(100.0)	0/1(0.0)	2/2(100.0)	0/2(0.0)
Total	**27/30(90.0)**	**3/30(10.0)**	**12/14(85.7)**	**2/14(14.3)**	**15/16(93.8)**	**1/16(6.3)**

*SEA=South East Asian
**N/A= Data not available
*** u/w = units per week

Table 4.12 Cross tabulation for human herpesvirus-8 in UK-oral lichen planus samples according to biopsy site, relevant medical note, corticosteroids, immune status, and therapies

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Biopsy site						
Buccal mucosa	22/25(88.0)	3/25(12.0)	9/11(81.8)	2/11(18.2)	12/13(92.3)	1/13(7.7)
Lt. of tongue*	3/3(100.0)	0/3(0.0)	2/2(100.0)	0/2(0.0)	1/1(100.0)	0/1(0.0)
Attached gingiva	2/2(100.0)	0/2(0.0)	1/1(100.0)	0/1(0.0)	2/2(100.0)	0/2(0.0)
Relevant medical note						
None	15/17(88.2)	2/17(11.8)	4/5(80.0)	1/5(20.0)	11/12(91.7)	1/12(8.3)
HRT**	1/2(50.0)	1/2(50.0)	1/2(50.0)	1/2(50.0)	-	-
Asthma	2/2(100.0)	0/2(0.0)	2/2(100.0)	0/2(0.0)	-	-
Candida	3/3(100.0)	0/3(0.0)	1/1(100.0)	0/1(0.0)	2/2(100.0)	0/2(0.0)
Cutaneous disease	4/4(100.0)	0/4(0.0)	2/2(100.0)	0/2(0.0)	2/2(100.0)	0/2(0.0)
Diabetes mellitus	2/2(100.0)	0/2(0.0)	2/2(100.0)	0/2(0.0)	-	-
Corticosteroids						
None	23/26(88.5)	3/26(11.5)	9/11(81.8)	2/11(18.2)	14/15(93.3)	1/15(6.7)
Topical	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Systemic	3/3(100.0)	0/3(0.0)	2/2(100.0)	0/2(0.0)	1/1(100.0)	0/1(0.0)
Immune status						
None	18/21(85.7)	3/21(14.3)	7/9(77.8)	2/9(22.2)	11/12(91.7)	1/12(8.3)
Systemic	4/4(100.0)	0/4(0.0)	2/2(100.0)	0/2(0.0)	2/2(100.0)	0/2(0.0)
Drugs	5/5(100.0)	0/5(0.0)	3/3(100.0)	0/3(0.0)	2/2(100.0)	0/2(0.0)
Therapies						
None	27/30(90.0)	3/30(10.0)	12/14(85.7)	2/14(14.3)	15/16(93.8)	1/16(6.3)
Total	**27/30(90.0)**	**3/30(10.0)**	**12/14(85.7)**	**2/14(14.3)**	**15/16(93.8)**	**1/16(6.3)**

* Lt. of tongue= lateral border of the tongue
** HRT= Hormone replacement therapy

Table 4.13 Human herpesvirus-8 infection in relation to UK-oral lichen planus samples

UK	Gender				Odds Ratio (95% CI)	P-value*
OLP						
	ALL			**OLP**		
				- +		
		HHV8	-	50 27	N/C	<0.001
			+	0 3		
	F			**OLP**		
				- +		
		HHV8	-	29 12	N/C	<0.001
			+	0 2		
	M			**OLP**		
				- +		
		HHV8	-	21 15	N/C	<0.001
			+	0 1		

N/C: could not be calculated because none of the normal controls were HHV-8 +ve
* Calculated from Fisher's exact test

4.4 Human herpesvirus- 8 in oral lichen planus of patients from UK

The frequency of human herpesvirus-8 DNA among oral lesions is summarised in Table 4.1. An overall HHV-8 DNA positive proportion of 3/30 (10.0%) was detected in OLP-UK patients. Demographic characteristics and risk factors for OLP in the UK patients are summarised in Table 4.11.

4.4.1 Gender

Human herpesvirus-8 DNA was detected in 2/14 (14.3%) of females compared with 1/16 (6.3%) in males.

4.4.2 Age groups

When HHV-8 DNA was examined in different age groups, the proportion of HHV-8 DNA was 1/5 (20.0%) in the 70-80 year age group, 1/10 (10.0%) in 50-60 year age group and 1/7 (14.3%) in 40-50 year age group. HHV-8 DNA was detected in 1/4 (25.0%) in females aged 40-50 years and in 1/5 (20.0%) in 50-60 year age group compared with 1/3 (33.3%) in males aged 70-80 years.

4.4.3 Ethnic origins

Human herpesvirus-8 DNA was detected in 1/8 (12.5%) of female Caucasians compared with 1/3 (33.3%) of female South East Asians. HHV-8 DNA was detected in 1/6 (16.7%) of the males with no recorded ethnicity data.

4.4.4 Association with tobacco, alcohol and Paan

The association between HHV-8 and other major risk factors for OLP (e.g. tobacco smoking, frequency of smoking, Paan use and alcohol intake)

was examined. Human herpesvirus-8 DNA was detected only in 3/26 (11.5%) of non-smokers. When the use of Paan was examined, HHV-8 DNA was found in 3/29 (10.3%) of non-Paan users. Human herpesvirus-8 DNA was detected in only 3/23 (13.0%) of non-alcohol drinkers.

4.4.5 Site of oral lichen planus smaples

Cross tabulation for HHV-8 DNA frequency and other characteristics are summarised in Table 4.12. Human herpesvirus-8 DNA was detected exclusively in the buccal mucosa.

4.4.6 Relevant medical history

Human herpesvirus-8 DNA was detected in 2/17 (11.8%) of patients with no relevant medical history. When stratified according to gender and medical history, HHV-8 was detected in 1/2 (50.0%) of female patients who had received HRT. The results show that none of the individuals infected with HHV-8 DNA had received any corticosteroid, chemo/radiotherapy or had any known immune diseases.

4.4.7 HHV-8 infection in relation to UK-OLP

The results for HHV-8 infection in relation to OLP are summarised in Table 4.13. In the sample of people, none of the normal controls was HHV-8+ve. However, some of the OLP patients were HHV-8 +ve. Thus the OLP patients were highly significantly more likely than normal controls to be HHV-8+ve in this sample ($P<0.001$, Fisher's exact test). Since none of the controls were HHV-8+ve, it was not possible to calculate an odds ratio. Therefore, it was also not possible to control for the effects of any of the risk factors in this analysis.

Table 4.14 Detection of human herpesvirus-8 versus demographics and risk factors for oral squamous cell carcinoma of UK patients with human imunodeficiency virus

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Gender						
Female	1/3(33.3)	2/3(66.7)				
Male	12/22(54.5)	10/22(45.5)				
Age (years)						
30-40	6/8(75.0)	2/8(25.0)	1/2(50.0)	1/2(50.0)	5/6(83.3)	1/6(16.7)
40-50	7/13(53.8)	6/13(46.2)	-	-	7/13(53.8)	6/13(46.2)
50-60	0/4(0.0)	4/4(100.0)	0/1(0.0)	1/1(100.0)	0/3(0.0)	3/3(100.0)
Ethnicity						
Caucasian	13/22(59.1)	9/22(40.9)	1/2(50.0)	1/2(50.0)	12/20(60.0)	8/20(40.0)
SEA*	0/1(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	-	-
African	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
N/A**	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Tobacco						
None	7/18(38.9)	11/18(61.1)	1/3(33.3)	2/3(66.7)	6/15(40.0)	9/15(60.0)
Current	6/7(85.7)	1/7(14.3)	-	-	6/7(85.7)	1/7(14.3)
Smoking						
None	7/18(38.9)	11/18(61.1)	1/3(33.3)	2/3(66.7)	6/15(40.0)	9/15(60.0)
>20/day	6/7(85.7)	1/7(14.3)	-	-	6/7(85.7)	1/7(14.3)
Paan						
None	13/25(52.0)	12/25(48.0)	1/3(33.3)	2/3(66.7)	12/22(54.5)	10/22(45.5)
Alcohol						
None	12/24(50.0)	12/24(50.0)	1/3(33.3)	2/3(66.7)	11/2152.4)	10/21(47.6)
>20 u/w***	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Total	**13/25(52.0)**	**12/25(48.0)**	**1/3(33.3)**	**2/3(66.7)**	**12/22(54.5)**	**10/22(45.5)**

*SEA=South East Asian
**N/A= Data not available
*** u/w = units per week

Table 4.15 Cross tabulation for human herpesvirus-8 in human imunodeficiency virus UK samples according to biopsy site, relevant medical note, corticosteroids, immune status, and therapies

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Biopsy site						
Buccal mucosa	3/4(75.0)	1/4(25.0)	-	-	3/4(75.0)	1/4(25.0)
Lt. of tongue*	0/2(0.0)	2/2(100.0)	-	-	0/2(0.0)	2/2(100.0)
Attached gingiva	0/2(0.0)	2/2(100.0)	-	-	0/2(0.0)	2/2(100.0)
Hard palate	2/5(40.0)	3/5(60.0)	0/2(0.0)	2/2(100.0)	2/3(66.7)	1/3(33.3)
Ant. 2/3 of tongue**	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Lip	6/6(100.0)	0/6(0.0)	1/1(100.0)	0/1(0.0)	5/5(100.0)	0/5(0.0)
Commisure	2/3(66.7)	1/3(33.3)	-	-	2/3(66.7)	1/3(33.3)
Retromolar	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Other ***	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Relevant medical note						
None	10/20(50.0)	10/20(50.0)	1/3(33.3)	2/3(66.7)	9/17(52.9)	8/17(47.1)
Candida	3/4(75.0)	1/4(25.0)	-	-	3/4(75.0)	1/4(25.0)
Cutaneous disease	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Corticosteroids						
None	13/18(72.2)	5/18(27.8)	1/2(50.0)	1/2(50.0)	12/16(75.0)	4/16(25.0)
Systemic	0/7(0.0)	7/7(100.0)	0/1(0.0)	1/1(100.0)	0/6(0.0)	6/6(100.0)
Immune status						
None	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Systemic	12/24(50.0)	12/24(50.0)	1/3(33.3)	2/3(66.7)	11/21(52.4)	10/21(47.6)
Therapies						
None	7/19(36.8)	12/19(63.2)	1/3(33.3)	2/3(66.7)	6/16(37.5)	10/16(62.5)
Triple therapy	6/6(100.0)	0/6(0.0)	-	-	6/6(100.0)	0/6(0.0)
Total	**13/25(52.0)**	**12/25(48.0)**	**1/3(33.3)**	**2/3(66.7)**	**12/22(54.5)**	**10/22(45.5)**

* Lt. of tongue= lateral border of the tongue
** Ant. 2/3 of tongue= anterior 2/3 of tongue
*** Other sites= Data not available

Table 4.16 Human herpesvirus-8 infection in relation to UK-human immunodeficiency virus samples

UK	Gender				Odds Ratio (95% CI)	P-value*
HIV						
	ALL		HIV -	HIV +		
		HHV8 -	50	13	N/C	<0.001
		HHV8 +	0	12		
	F		HIV -	HIV +		
		HHV8 -	29	1	N/C	<0.001
		HHV8 +	0	2		
	M		HIV -	HIV +		
		HHV8 -	21	12	N/C	<0.001
		HHV8 +	0	10		

N/C: could not be calculated because none of the normal controls were HPV +ve
* Calculated from Fisher's exact test

4.5 Human herpesvirus- 8 in HIV individuals-UK

The frequency of human herpesvirus-8 DNA among oral lesions is summarised in Table 4.1. An overall HHV-8 DNA positive proportion of 12/25 (48.0%) was detected in HIV-UK patients. Demographic characteristics and risk factors for HIV in the UK patients are summarised in Table 4.14.

4.5.1 Gender

Human herpesvirus-8 DNA was detected in 2/3 (66.7%) of females compared with 10/22 (45.5%) in males.

4.5.2 Age groups

When HHV-8 DNA was examined in different age groups, the proportion was 2/8 (25.0%) in the 30-40 year age group, 6/13 (46.2%) in the 40-50 year age group and in 4/4 (100.0%) in the 50-60 year age group. When stratified according to gender and age groups, HHV-8 DNA was found in 1/2 (50.0%) of the female group aged 30-40 years compared with 1/6 (16.7%) of males within the same age group. Human herpesvirus-8 DNA was detected in 1/1 (100.0%) of the female group aged 50-60 years compared with 3/3 (100.0%) of male within the same age group. In addition, HHV-8 was positive in 6/13 (46.2%) of the male group aged 40-50 years.

4.5.3 Ethnic origins

Human herpesvirus-8 DNA was detected in 9/22 (40.9%) of Caucasians compared with 1/1 (100.0%) in Africans. In addition, HHV-8 was detected in other ethnic groups e.g. in 1/1 (100.0%) South East Asian. When stratified according to gender and ethnicity, HHV-8 DNA was found in 1/2 (50.0%) of Caucasian females compared with 8/20 (40.0%) in male

Caucasians. However, HHV-8 DNA was found in 1/1 (100.0%) of male patient with no recorded ethnicity.

4.5.4 Association with tobacco, alcohol and Paan

The association between HHV-8 and other major risk factors for HIV (e.g. tobacco smoking, frequency of smoking, Paan use and alcohol intake) was examined. Human herpesvirus-8 DNA was found in 11/18 (61.1%) of non-smokers compared with 1/7 (14.3%) of males currently smoking >20/day. When the use of Paan was examined, HHV-8 DNA was found only in 12/25 (48.0%) of non-Paan users. The rate of HHV-8 DNA was detected in 12/24 (50.0%) of non-alcohol drinkers.

4.5.5 Site of human immunodeficiency virus sample

Cross tabulation for HHV-8 DNA frequency and other characteristics are summarised in Table 4.15. Human herpesvirus-8 DNA was detected in the hard palate, lateral border of the tongue, attached gingiva and, to a lesser extent, the buccal mucosa, anterior two thirds of tongue, commisure, and retromolar area. No difference was readily apparent as regards the biopsy site and HHV-8 infection.

4.5.6 Relevant medical history

Human herpesvirus-8 DNA was detected in 10/20 (50.0%) of patients with no relevant medical history. In addition, HHV-8 DNA was found in 1/4 (25.0%) of males with candidal infection and in 1/1 (100.0%) male with a cutaneous disease. The results show that HHV-8 DNA was found in 5/18 (27.8%) of individuals who had received no corticosteroid therapy compared with 7/7 (100.0%) of patients who received systemic corticosteroids. Human

herpesvirus-8 was found in 12/24 (50.0%) of patients with systemic immune disease. When further stratified according to gender and immune status, HHV-8 DNA was detected in 2/3 (66.7%) of female patients compared with 10/21 (47.6%) in male individuals. No HHV-8 DNA was detected in patients who received triple therapy. In addition, HHV-8 DNA was detected in 12/19 (63.2%) of patients received no therapy.

4.5.7 HHV-8 infection in relation to UK-HIV

The results for HHV-8 infection in relation to HIV are summarised in Table 4.16. In the sample of people, none of the normal controls was HHV-8+ve. However, some of the HIV patients were HHV-8 +ve. Thus the HIV patients were highly significantly more likely than normal controls to be HHV-8+ve in this sample ($P<0.001$, Fisher's exact test). However, since none of the controls were HHV-8+ve, it was not possible to calculate an odds ratio. Therefore, it was also not possible to control for the effects of any of the risk factors in this analysis.

4.6 Human herpesvirus-8 in normal control

None of the normal controls had detectable HHV-8. Thus, the demographic characteristics of this sample are as has already been described in the previous chapter (See tables 3.17 and 3.18)

Table 4.17 Detection of human herpesvirus-8 versus demographics and risk factors for oral squamous cell carcinoma of KSA patients with oral squamous cell carcinoma

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Gender						
Female	6/7(85.7)	1/7(14.3)				
Male	17/23(73.9)	6/23(26.1)				
Age (years)						
30-40	2/2(100.0)	0/2(0.0)	-	-	2/2(100.0)	0/2(0.0)
40-50	2/2(100.0)	0/2(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)
50-60	5/7(71.4)	2/7(28.6)	0/1(0.0)	1/1(100.0)	5/6(83.3)	1/6(16.7)
60-70	7/11(63.6)	4/11(36.4)	2/2(100.0)	0/2(0.0)	5/9(55.6)	4/9(44.4)
70-80	2/3(66.7)	1/3(33.3)	-	-	2/3(66.7)	1/3(33.3)
>80	5/5(100.0)	0/5(0.0)	3/3(100.0)	0/3(0.0)	2/2(100.0)	0/2(0.0)
Ethnicity						
Caucasian	1/2(50.0)	1/2(50.0)	-	-	1/2(50.0)	1/2(50.0)
Arab	22/28(78.6)	6/28(21.4)	6/7(85.7)	1/7(14.3)	16/21(76.2)	5/21(23.8)
Tobacco						
None	23/29(78.3)	6/29(20.7)	6/7(85.7)	1/7(14.3)	17/22(77.3)	5/22(22.7)
Current	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Smoking						
None	23/29(78.3)	6/29(20.7)	6/7(85.7)	1/7(14.3)	17/22(77.3)	5/22(22.7)
10-15/day	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Paan						
None	23/30(76.7)	7/30(23.3)	6/7(85.7)	1/7(14.3)	17/23(73.9)	6/23(26.1)
Alcohol						
None	23/30(76.7)	7/30(23.3)	6/7(85.7)	1/7(14.3)	17/23(73.9)	6/23(26.1)
Total	**23/30(76.7)**	**7/30(23.3)**	**6/7(85.7)**	**1/7(14.3)**	**17/23(73.9)**	**6/23(26.1)**

Table 4.18 Cross tabulation for human herpesvirus-8 in KSA-oral squamous cell carcinoma samples according to biopsy site, relevant medical note, corticosteroids, immune status and therapies

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Biopsy site						
Buccal mucosa	3/6(50.0)	3/6(50.0)	2/2(100.0)	0/2(0.0)	1/4(24.0)	3/4(75.0)
Lt. of tongue*	6/7(85.7)	1/7(14.3)	1/1(100.0)	0/1(0.0)	5/6(83.3)	1/6(16.7)
Floor of mouth	2/2(100.0)	0/2(0.0)	-	-	2/2(100.0)	0/2(0.0)
Ant. 2/3 of tongue**	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Lip	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Retromolar	8/8(100.0)	0/8(0.0)	1/1(100.0)	0/1(0.0)	7/7(100.0)	0/7(0.0)
Other***	2/5(40.0)	3/5(60.0)	1/2(50.0)	1/2(50.0)	1/3(33.3)	2/3(66.7)
Relevant medical note						
None	19/25(76.0)	6/25(24.0)	4/5(80.0)	1/5(20.0)	15/20(75.0)	5/20(25.0)
SCC****	1/2(50.0)	1/2(50.0)	1/1(100.0)	0/1(0.0)	0/1(0.0)	1/1(100.0)
Diabetes mellitus	2/2(100.0)	0/2(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)
Penicillin allergy	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Corticosteroids						
None	23/30(76.7)	7/30(23.3)	6/7(85.7)	1/7(14.3)	17/23(73.9)	6/23(26.1)
Immune status						
None	23/30(76.7)	7/30(23.3)	6/7(85.7)	1/7(14.3)	17/23(73.9)	6/23(26.1)
Therapies						
None	23/30(76.7)	7/30(23.3)	6/7(85.7)	1/7(14.3)	17/23(73.9)	6/23(26.1)
Total	**23/30(76.7)**	**7/30(23.3)**	**6/7(85.7)**	**1/7(14.3)**	**17/23(73.9)**	**6/23(26.1)**

* Lt. of tongue= lateral border of the tongue
** Ant. 2/3 of tongue= anterior 2/3 of tongue
*** Other sites= Data not available
**** SCC= Second primary Squamous Cell Carcinoma

Table 4.19 Human herpesvirus-8 infection in relation to KSA-oral squamous cell carcinoma samples

KSA	Gender				Odds Ratio (95% CI)	P-value*
OSCC						
	ALL		**OSCC**			
			-	+		
		HHV8 -	50	23	N/C	<0.001
		HHV8 +	0	7		
	F		**OSCC**			
			-	+		
		HHV8 -	29	6	N/C	<0.001
		HHV8 +	0	1		
	M		**OSCC**			
			-	+		
		HHV8 -	21	17	N/C	<0.001
		HHV8 +	0	6		

N/C: could not be calculated because none of the normal controls were HHV-8 +ve
* Calculated from Fisher's exact test

4.7 Human herpesvirus- 8 in oral squamous cell carcinoma - KSA

The frequency of human herpesvirus-8 DNA among oral lesions is summarised in Table 4.1. An overall HHV-8 DNA positive proportion of 7/30 (23.3%) was detected in OSCC-KSA patients. Demographic characteristics and risk factors for OSCC in the KSA patients are summarised in Table 4.17

4.7.1 Gender

Human herpesvirus-8 DNA was detected in 1/7 (14.3%) of females compared with 6/23 (26.1%) in males.

4.7.2 Age groups

When HHV-8 DNA was examined in different age groups, HHV-8 DNA was 2/7 (28.6%) in the 50-60 year age group compared with 4/11 (36.4%) in the 60-70 year age group. When stratified according to gender and age groups, HHV-8 DNA was found in 1/1 (100.0%) of females aged 50-60 years compared with 1/6 (16.7%%5) of males within the same age group. HHV-8 was found in 4/9 (44.4%) of males in the 60-70 year age group.

4.7.3 Ethnic origins

Human herpesvirus-8 DNA was detected in 1/2 (50.0%) of male Caucasians compared with 5/21 (23.8%) Arab males. HHV-8 DNA was detected in 1/7 (14.3%) of Arab females. There were no Caucasian females in the sample.

4.7.4 Association with tobacco, alcohol and Paan

The association between HHV-8 and other major risk factors for OSCC (e.g. tobacco smoking, frequency of smoking, Paan use and alcohol

intake) were examined. Human herpesvirus-8 DNA was detected in 6/29 (20.7%) of non-smokers compared with 1/1 (100.0%) current smoker male smoking 10-15/day. There were no Paan users in the sample when the use of Paan was examined, HHV-8 DNA was found in 7/30 (23.3%) of non-Paan users. In addition, HHV-8 DNA was detected in 7/30 (23.3%) of non-alcohol drinkers only. Similarly, none of the sample reported that they drink alcohol.

4.7.5 Site of oral squamous cell carcinoma samples

Cross tabulation for HHV-8 DNA frequency and other characteristics are summarised in Table 4.18. Although, only small numbers were present in the samples, HHV-8 DNA was detected in the buccal mucosa and lateral border of the tongue. HHV-8 was detected in 3/5 (60.0%) of patients with no record of sample site. When stratified according to gender and biopsy site, HHV-8 was found in 1/2 (50.0%) of females and in 2/3 (66.7%) of males with no recorded data of biopsy site. HHV-8 was detected in 3/4 (75.0%) of the buccal mucosa in males.

4.7.6 Relevant medical history

Human herpesvirus-8 DNA was detected in 1/2 (50.0%) of patients with second primary OSCC compared with 6/25 (24.0%) of patients with no relevant medical history. When stratified according to gender and medical history, HHV-8 was detected in 1/5 (20.0%) of female patients with second primary OSCC compared with 5/20 (25.0%) of male within the same group. The results show that none of the individuals infected with HHV-8 DNA had received any corticosteroids, chemo/radiotherapy or had any immune diseases.

4.7.7 HHV-8 infection in relation to KSA-OSCC

The results for HHV-8 infection in relation to OSCC are summarised in Table 4.19. In the sample of people, none of the normal controls was HHV-8+ve. However, some of the OSCC patients were HHV-8 +ve. Thus the OSCC patients were highly significantly more likely than normal controls to be HHV-8+ve in this sample ($P<0.001$, Fisher's exact test). Since none of the controls were HHV-8+ve, it was not possible to calculate an odds ratio. Therefore, it was also not possible to control for the effects of any of the risk factors in this analysis.

Table 4.20 Detection of human herpesvirus-8 versus demographics and risk factors for oral squamous cell carcinoma of KSA patients withoral lichen planus

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Gender						
Female	11/19(57.9)	8/19(42.1)				
Male	9/13(69.2)	4/13(30.8)				
Age (years)						
20-30	2/3(66.7)	1/3(33.3)	1/1(100.0)	0/1(0.0)	1/2(50.0)	1/2(50.0)
30-40	5/7(71.4)	2/7(28.6)	4/6(66.7)	2/6(33.3)	1/1(100.0)	0/1(0.0)
40-50	5/9(55.6)	4/9(44.4)	3/7(42.9)	4/7(57.1)	2/2(100.0)	0/2(0.0)
50-60	4/8(50.0)	4/8(50.0)	1/2(50.0)	1/2(50.0)	3/6(50.0)	3/6(50.0)
60-70	4/5(80.0)	1/5(20.0)	2/3(66.7)	1/3(33.3)	2/2(100.0)	0/2(0.0)
Ethnicity						
SEA*	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
FEA**	1/2(50.0)	1/2(50.0)	-	-	1/2(50.0)	1/2(50.0)
Arab	18/29(62.1)	11/29(37.9)	11/19(57.9)	8/19(42.1)	7/10(70.0)	3/10(30.0)
Tobacco						
None	20/32(62.5)	12/32(37.5)	11/19(57.9)	8/19(42.1)	9/13(69.2)	4/13(30.8)
Smoking						
None	20/32(62.5)	12/32(37.5)	11/19(57.9)	8/19(42.1)	9/13(69.2)	4/13(30.8)
Paan						
None	20/32(62.5)	12/32(37.5)	11/19(57.9)	8/19(42.1)	9/13(69.2)	4/13(30.8)
Alcohol						
None	20/32(62.5)	12/32(37.5)	11/19(57.9)	8/19(42.1)	9/13(69.2)	4/13(30.8)
Total	**20/32(62.5)**	**12/32(37.5)**	**11/19(57.9)**	**8/19(42.1)**	**9/13(69.2)**	**4/13(30.8)**

*SEA=South East Asian
** FEA= Far East Asia

Table 4.21 Cross tabulation for human herpesvirus-8 in KSA-oral lichen planus samples according to biopsy site, relevant medical note, corticosteroids, immune status, and therapies

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Biopsy site						
Buccal mucosa	16/27(59.3)	11/27(40.7)	9/17(52.9)	8/17(47.1)	7/10(70.0)	3/10(30.0)
Lt. of tongue*	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Attached gingiva	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Ant. 2/3 of tongue**	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Lip	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Other***	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Relevant medical note						
None	15/20(75.0)	5/20(25.0)	8/12(66.7)	4/12(33.3)	7/8(87.5)	1/8(12.5)
Asthma	0/1(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	-	-
Cutaneous disease	2/6(33.3)	4/6(66.7)	1/3(33.3)	2/3(66.7)	1/3(33.3)	2/3(66.7)
Diabetes mellitus	3/5(60.0)	2/5(40.0)	2/3(66.7)	1/3(33.3)	1/2(50.0)	1/2(50.0)
Corticosteroids						
None	20/32(62.5)	12/32(37.5)	11/19(57.9)	8/19(42.1)	9/13(69.2)	4/13(30.8)
Immune status						
None	20/32(62.5)	12/32(37.5)	11/19(57.9)	8/19(42.1)	9/13(69.2)	4/13(30.8)
Therapies						
None	20/32(62.5)	12/32(37.5)	11/19(57.9)	8/19(42.1)	9/13(69.2)	4/13(30.8)
Total	**20/32(62.5)**	**12/32(37.5)**	**11/19(57.9)**	**8/19(42.1)**	**9/13(69.2)**	**4/13(30.8)**

* Lt. of tongue= lateral border of the tongue
** Ant. 2/3 of tongue= anterior 2/3 of tongue
*** Other sites= Data not available

Table 4.22 Human herpesvirus-8 infection in relation to KSA-oral lichen planus samples

KSA	Gender			OLP -	OLP +	Odds Ratio (95% CI)	P-value*
OLP							
	ALL						
		HHV8	-	50	20	N/C	<0.001
			+	0	12		
	F						
		HHV8	-	29	11	N/C	<0.001
			+	0	3		
	M						
		HHV8	-	21	9	N/C	<0.001
			+	0	4		

N/C: could not be calculated because none of the normal controls were HHV-8 +ve
* Calculated from Fisher's exact test

4.8 Human herpesvirus- 8 in oral lichen planus - KSA

The frequency of human herpsevirus-8 DNA among oral lesions is summarised in Table 4.1. An overall HHV-8 DNA positive proportion of 12/32 (37.5%) was detected in OLP-KSA patients. Demographic characteristics and risk factors for OLP in the KSA pateints are summarised in Table 4.20

4.8.1 Gender

Human herpesvirus-8 DNA was detected in 8/19 (42.1%) of females compared with 4/13 (30.8%) in males.

4.8.2 Age groups

When HHV-8 DNA was examined in different age groups, the proportion was found in 1/3 (33.3%) of the 20-30 year age group, 2/7 (28.6%) of the 30-40 year age group, 4/9 (44.4%) of the 40-50 year age group and 4/8 (50.0%) of the 50-60 year age group. In addition, HPV-DNA was found in 1/5 (20.0%) in the 60-70 year age group. When stratified according to gender and age groups. Human herpesvirus-8 DNA was found in 1/2 (50.0%) of the female group aged 50-60 compared with 3/6 (50.0%) of males within the same age group.

4.8.3 Ethnic origins

Human herpesvirus-8 DNA was detected in 11/29 (37.9%) of Arabs compared with 1/2 (50.0%) of Far East Asians. When stratified according to gender and ethnicity, HHV-8 DNA was found in 8/19 (42.1%) of Arab females compared with 3/10 (30.0%) of males within the same ethnic group.

4.8.4 Association with tobacco, alcohol and Paan

The association between HHV-8 and other major risk factors for OLP (e.g. tobacco smoking, frequency of smoking, Paan use and alcohol intake) was examined. Human herpesvirus-8 DNA was found in 12/32 (37.5%) of non-smokers. When the use of Paan was examined, HHV-8 DNA was found in only 12/32 (37.5%) of non-Paan users. The rate of HHV-8 DNA was detected in 12/32 (37.5%) of non-alcohol drinkers. None of the sample reported having tobacco, alcohol or Paan habits.

4.8.5 Site of oral lichen planus samples

Cross tabulation for HHV-8 DNA frequency and other characteristics are summarised in Table 4.21. Human herpesvirus-8 DNA was detected in the buccal mucosa and in the anterior two thirds of the tongue. When further stratified according to gender and biopsy site, HHV-8 DNA was found in the buccal mucosa of 3/10 (30.0%) of males compared with 8/17 (47.1%) females within the same group.

4.8.6 Relevant medical history

Human herpesvirus-8 DNA was found in 5/20 (25.0%) of patients with no relevant medical history and 4/6 (66.7%) of patients with a cutaneous disease. In addition, HHV-8 was found in 1/2 (50.0%) of patients with asthma and in 2/5 (40.0%) of patients with diabetes. When further stratified according to gender and any relevant medical history, HHV-8 was detected in 4/12 (33.3%) of female patients with no relevant medical history and in 1/8 (12.5%) of male patients within the same group. HHV-8 was found in 2/3 (66.7%) of patients with a cutaneous disease in both genders.

The results show that HHV-8 DNA was found in 12/32 (37.5%) of patients who had received no corticosteroids or with any immune diseases. None of the patients who detected positive for HHV-8 DNA had received chemotherapy or radiotherapy.

4.8.7 HHV-8 infection in relation to KSA-OLP

The results for HHV-8 infection in relation to OLP are summarised in Table 4.22 In the sample of people, none of the normal controls was HHV-8+ve. However, some of the OLP patients were HHV-8 +ve. Thus the OLP patients were highly significantly more likely than normal controls to be HHV-8+ve in this sample ($P<0.001$, Fisher's exact test). Since none of the controls were HHV-8+ve, it was not possible to calculate an odds ratio. Therefore, it was also not possible to control for the effects of any of the risk factors in this analysis.

Table 4.23 Detection of human herpesvirus-8 versus demographicss and risk factors for oral squamous cell carcinoma of KSA patients with salivary gland lesions

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Gender						
Female	5/7(71.4)	2/7(28.6)				
Male	10/13(76.9)	3/13(23.1)				
Age (years)						
20-30	2/3(66.7)	1/3(33.3)	0/1(0.0)	1/1(100.0)	2/2(100.0)	0/2(0.0)
30-40	4/5(80.0)	1/5(20.0)	1/1(100.0)	0/1(0.0)	3/4(75.0)	1/4(25.0)
40-50	4/5(80.0)	1/5(20.0)	2/3(66.7)	1/3(33.3)	2/2(100.0)	0/2(0.0)
50-60	2/4(50.0)	2/4(50.0)	1/1(100.0)	0/1(0.0)	1/3(33.3)	2/3(66.7)
60-70	3/3(100.0)	0/3(0.0)	1/1(100.0)	0/1(0.0)	2/2(100.0)	0/2(0.0)
Ethnicity						
SEA*	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
FEA**	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Arab	13/18(72.2)	5/18(27.8)	5/7(71.4)	2/7(28.6)	8/11(72.7)	3/11(27.3)
Tobacco						
None	15/20(75.0)	5/20(25.0)	5/7(71.4)	2/7(28.6)	10/13(76.9)	3/13(23.1)
Smoking						
None	15/20(75.0)	5/20(25.0)	5/7(71.4)	2/7(28.6)	10/13(76.9)	3/13(23.1)
Paan						
None	15/20(75.0)	5/20(25.0)	5/7(71.4)	2/7(28.6)	10/13(76.9)	3/13(23.1)
Alcohol						
None	15/20(75.0)	5/20(25.0)	5/7(71.4)	2/7(28.6)	10/13(76.9)	3/13(23.1)
Total	**15/20(75.0)**	**5/20(25.0)**	**5/7(71.4)**	**2/7(28.6)**	**10/13(76.9)**	**3/13(23.1)**

*SEA=South East Asian
** FEA= Far East Asian

Table 4.24 Cross tabulation for human herpesvirus-8 in KSA salivary gland samples according to biopsy site, relevant medical note, corticosteroids, immune status and therapies

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Biopsy site						
Buccal mucosa	3/5(60.0)	2/5(40.0)	0/1(0.0)	1/1(100.0)	3/4(75.0)	1/4(25.0)
Floor of tongue	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Hard palate	8/9(88.9)	1/9(11.1)	3/3(100.0)	0/3(0.0)	5/6(83.3)	1/6(16.7)
Lip	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Retromolar	1/2(50.0)	1/2(50.0)	1/1(100.0)	0/1(0.0)	0/1(0.0)	1/1(100.0)
Other *	1/2(50.0)	1/2(50.0)	0/1(0.0)	1/1(100.0)	1/1(100.0)	0/1(0.0)
Relevant medical note						
None	15/20(75.0)	5/20(25.0)	5/7(71.4)	2/7(28.6)	10/13(76.9)	3/13(23.1)
Corticosteroids						
None	15/20(75.0)	5/20(25.0)	5/7(71.4)	2/7(28.6)	10/13(76.9)	3/13(23.1)
Immune status						
None	15/20(75.0)	5/20(25.0)	5/7(71.4)	2/7(28.6)	10/13(76.9)	3/13(23.1)
Therapies						
None	15/20(75.0)	5/20(25.0)	5/7(71.4)	2/7(28.6)	10/13(76.9)	3/13(23.1)
Total	**15/20(75.0)**	**5/20(25.0)**	**5/7(71.4)**	**2/7(28.6)**	**10/13(76.9)**	**3/13(23.1)**

* Other sites= Data not available

Table 4.25 Human herpesvirus-8 infection in relation to KSA salivary gland samples

KSA	Gender				Odds Ratio (95% CI)	P-value*
Salivary						
	ALL		**Salivary**			
			-	+		
		HHV8 -	50	15	N/C	<0.001
		HHV8 +	0	5		
	F		**Salivary**			
			-	+		
		HHV8 -	29	5	N/C	<0.001
		HHV8 +	0	2		
	M		**Salivary**			
			-	+		
		HHV8 -	21	10	N/C	<0.001
		HHV8 +	0	3		

N/C: could not be calculated because none of the normal controls were HHV-8 +ve
* Calculated from Fisher's exact test

4.9Human herpesvirus-8 in salivary gland lesions of patients from KSA

The frequency of human herpesvirus-8 DNA among oral lesions is summarised in Table 4.1. An overall HHV-8 DNA positive proportion of 5/25 (25.0%) was detected in salivary gland lesions-KSA patients. Demographic characteristics and risk factors for salivary gland lesions in the KSA patients are summarised in Table 4.23

4.9.1 Gender

Human herpesvirus-8 DNA was detected in 2/7 (28.6%) of females compared with 3/13 (23.1%) in males.

4.9.2 Age groups

When HHV-8 DNA was examined in different age groups, the rate was 1/1 (100.0%) in the 20-30 year age group and 1/3 (33.3%) in the 40-50 year age group of females and in 2/3 (66.7%) in the 50-60 year age group of males.

4.9.3 Ethnic origins

Human herpesvirus-8 DNA was detected in 5/18 (27.8%) of Arabs. When stratified according to gender and ethnicity, HHV-8 DNA was found in 2/7 (28.6%) of Arab females compared with 3/11 (27.3%) of male within the same ethnic group.

4.9.4 Association with tobacco, alcohol and Paan

The association between HHV-8 and other major risk factors for salivary samples (e.g. tobacco smoking, frequency of smoking, Paan use and alcohol intake) was examined. Human herpesvirus-8 DNA was found in 5/20

(25.0%) of non-smokers. None of the sample reported having tobacco, alcohol and Paan habits.

4.9.5 Site of salivary gland samples

Cross tabulation for HHV-8 DNA frequency and other characteristics are summarised in Table 4.24. Human herpesvirus-8 DNA was found in the buccal mucosa, hard palate and retromolar area. No difference was readily apparent as regards the biopsy site and HHV-8 infection, similar proportions were found in both genders.

4.9.6 Relevant medical history

The results show that none of the individuals infected with HHV-8 DNA had received any corticosteroid therapy or had any systemic immune disease. In addition, HHV-8 DNA was detected in patients who received no therapy.

4.9.7 HHV-8 infection in relation to KSA-Salivary gland lesions

The results for HHV-8 infection in relation to salivary samples are summarised in Table 4.25. In the sample of people, none of the normal controls was HHV-8+ve. However, some of the salivary gland lesion patients were HHV-8 +ve. Thus the salivary gland lesion patients were highly significantly more likely than normal controls to be HHV-8+ve in this sample ($P<0.001$, Fishers exact test). Since none of the controls were HHV-8+ve, it was not possible to calculate an odds ratio. Therefore, it was also not possible to control for the effects of any other risk factors in this analysis.

Chapter 5

Epstein-Barr virus Results

Table 5.1 Frequency of Epstein-Barr virus in examined oral pathologies

Country	Lesion	Gender	-ve No. (%)	+ve No. (%)
UK				
	PVL	All	6/12 (50.0)	6/12 (50.0)
		F	3/6 (50.0)	3/6 (50.0)
		M	3/6 (50.0)	3/6 (50.0)
	OED	All	44/45 (97.8)	1/45 (2.2)
		F	22/22 (100.0)	-
		M	22/23 (95.70)	1/23 (4.3)
	Normal control	All	44/50(88.0)	6/50(12.0)
		F	26/29(89.7)	3/29(10.3)
		M	18/21(85.7)	3/21(14.3)

PVL = Proliferative verrucous leukoplakia
OED = Oral epithelial dysplasia

Table 5.2 Detection of Epstein-Barr virus versus demographics and risk factors for oral squamous cell carcinoma of UK patients with proliferative verrucous leukoplakia

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Gender						
Female	3/6(50.0)	3/6(50.0)				
Male	3/6(50.0)	3/6(50.0)				
Age (years)						
40-50	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
50-60	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
60-70	2/3(66.7)	1/3(33.3)	1/2(50.0)	1/2(50.0)	1/1(100.0)	0/1(0.0)
70-80	2/6(33.3)	4/6(66.7)	1/3(33.3)	2/3(66.7)	1/3(33.3)	2/3(66.7)
>80	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Ethnicity						
Caucasian	1/5(20.0)	4/5(80.0)	1/4(25.0)	3/4(75.0)	0/1(0.0)	1/1(100.0)
N/A*	5/7(71.4)	2/7(28.6)	2/2(0.0)	0/2(0.0)	3/5(60.0)	2/5(40.0)
Tobacco						
None	6/10(60.0)	4/10(40.0)	3/6(50.0)	3/6(50.0)	3/4(75.0)	1/4(25.0)
Current	0/2(0.0)	2/2(100.0)	-	-	0/2(0.0)	2/2(100.0)
Smoking						
None	6/10(60.0)	4/10(40.0)	3/6(50.0)	3/6(50.0)	3/4(75.0)	1/4(25.0)
>20/day	0/2(0.0)	2/2(100.0)	-	-	0/2(0.0)	2/2(100.0)
Paan						
None	6/11(54.0)	5/11(45.5)	3/6(50.0)	3/6(50.0)	3/5(60.0)	2/5(40.0)
Current	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Alcohol						
None	6/11(54.0)	5/11(45.5)	3/6(50.0)	3/6(50.0)	3/5(60.0)	2/5(40.0)
>20 u/ w**	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Total	**6/12(50.0)**	**6/12(50.0)**	**3/6(50.0)**	**3/6(50.0)**	**3/6(50.0)**	**3/6(50.0)**

*N/A= Data not available
** u/w= units per week

Table 5.3 Cross tabulation for Epstein-Barr virus in UK-proliferative verrucous leukoplakia samples according to biopsy site, relevant medical note, corticosteroids, immune status and therapies

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Biopsy site						
Buccal mucosa	2/4(50.0)	2/4(50.0)	2/4(50.0)	2/4(50.0)	-	-
Lt. of tongue*	1/2(50.0)	1/2(50.0)	-	-	1/2(50.0)	1/2(50.0)
Floor of mouth	0/2(0.0)	2/2(100.0)	-	-	0/2(0.0)	2/2(100.0)
Hard palate	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Ant .2/3 of tongue**	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Lip	0/1(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	-	-
Retromolar	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Relevant medical note						
None	3/3(100.0)	0/3(0.0)	2/2(100.0)	0/2(0.0)	1/1(100.0)	0/1(0.0)
SCC***	3/6(50.0)	3/6(50.0)	1/3(33.3)	2/3(66.7)	2/3(66.7)	1/3(33.3)
Candidal infection	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Penicillin allergy	0/2(0.0)	2/2(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)
Corticosteroids						
None	6/12(50.0)	6/12(50.0)	3/6(50.0)	3/6(50.0)	3/6(50.0)	3/6(50.0)
Immune status						
None	6/12(50.0)	6/12(50.0)	3/6(50.0)	3/6(50.0)	3/6(50.0)	3/6(50.0)
Therapies						
None	6/11(54.5)	5/11(45.5)	3/5(60.0)	2/5(40.0)	3/6(50.0)	3/6(50.0)
Chemotherapy	0/1(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	-	-
Total	**6/12(50.0)**	**6/12(50.0)**	**3/6(50.0)**	**3/6(50.0)**	**3/6(50.0)**	**3/6(50.0)**

* Lt. of tongue= lateral border of the tongue
** Ant. 2/3 of tongue= anterior 2/3 of tongue
*** SCC= Second primary Squamous Cell Carcinoma

Table 5.4 Epstein-Barr virus infection in relation to UK proliferative verrucous leukoplakia

UK	Gender				Crude Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)
PVL						
	All		PVL -	PVL +		
		EBV -	44	6	7.33(1.78,30.25)	173.34(2.64,11387.41)*
		EBV +	6	6		
	F		PVL -	PVL +		
		EBV -	26	3	8.67(1.18,63.87)	N/C¶
		EBV +	3	3		
	M		PVL -	PVL +		
		EBV -	18	3	6.00(0.08,44.95)	N/C¶
		EBV +	3	3		

* Adjusted for age, ethnicity, smoking, Paan and alcohol
¶ N/C= OR could not be calculated because some of the potential explanatory factors exhibit no variation.

5.1 Epstein-Barr virus in proliferative verrucous leukoplakia - UK

The frequency of Epstein-Barr virus DNA among oral lesions is summarised in Table 5.1. An overall EBV-DNA positive proportion of 6/12 (50.0%) was detected in PVL-UK patients. Demographic characteristics and risk factors for PVL in the UK pateints are summarised in Table 5.2.

5.1.1 Gender

Epstein-Barr virus DNA was detected in 3/6 (50.0%) of both genders.

5.1.2 Age groups

When EBV-DNA was examined in different age groups, EBV infection was 1/1 (100.0%) in the 50-60 year age group, 1/3 (33.3%) in the 60-70 year age groups and 4/6 (66.7%) in the 70-80 year age groups. When stratified according to gender and age, EBV-DNA was detected in 1/2 (50.0%) of the female aged 60-70 years and in 2/3 (66.7%) of the females aged 70-80 years, while EBV-DNA was found in 1/1 (100.0%) of the males aged 50-60 years and in 2/3 (66.7%) of males aged 70-80 years.

5.1.3 Ethnic origins

Epstein-Barr virus DNA was detected in 4/5 (80.0%) of Caucasians compared with 2/7 (28.5%) of cases with no recorded ethnicity. When stratified according to gender and ethnicity, 3/4 (75.0%) of the Caucasian females were EBV positive compared with 1/1 (100.0%) of Caucasians male were EBV positive, and 2/5 (40.0%) of male patients with no recorded ethnicity were EBV infected. Neither of the two females 0/2 (0.0%) of unknown ethnicities were EBV+ve.

5.1.4 Association with tobacco, alcohol and Paan

The association between EBV and other major risk factors (e.g. tobacco smoking, frequency of smoking, Paan use and alcohol intake) was examined. Epstein-Barr virus DNA was detected in 2/2 (100.0%) of current smokers compared with 4/10 (40.0%) of non-smokers. Among the smokers group, EBV-DNA was detected in 2/2 (100.0%) of individuals smoking > 20/day. When stratified according to gender and smoking, none of the females in this group were current smokers. Epstein-Barr virus DNA was detected in 1/4 (25.0%) of male non-smokers compared with 2/2 (100.0%) of males smoking >20/day.

When the use of Paan was examined, EBV-DNA was found in 5/11 (45.5%) of non-Paan users compared with 1/1 (100.0%) of Paan users. When stratified according to gender and use of Paan, EBV-DNA was detected in 3/6 (50.0%) of female non-Paan user compared with 2/5(40.0%) of male non-Paan user. EBV-DNA was found in 1/1 (100.0%) male Paan user. There were no females Paan users in the sample.

Epstein-Barr virus DNA was detected in 5/11 (45.0%) of non-alcohol drinkers compared with 1/1 (100.0%) of individuals currently consuming >20 units per week. When stratified according to gender and alcohol intake, the results show that EBV was detected in 3/6 (50.0%) of female non-alcohol drinkers compared with 2/5 (40.0%) of males within the same group. Among the male alcohol-drinking group, EBV-DNA was found in one of males consuming > 20 units per week. No females reported that they consumed alcohol.

5.1.5 Site of proliferative verrucous leukoplakia samples

Cross tabulation for EBV-DNA frequency and other characteristics are summarised in Table 5.3. Epstein-Barr virus DNA was found in the buccal mucosa, lateral border of the tongue, floor of mouth and on the lip. No difference was readily apparent as regards the biopsy site and EBV infection.

5.1.6 Relevant medical history

Epstein-Barr virus DNA was detected in 3/6 (50.0%) of patients with second primary OSCC. EBV-DNA was found in 1/1 (100.0%) of males with candidal infection and in 2/2 (100.0%) of individuals allergic to penicillin. When stratified according to gender and medical history, EBV-DNA was found in 2/3 (66.7%) of females with second primary OSCC compared with 1/3 (33.3%) of males within the same group. EBV-DNA was detected in 1/1 (100.0%) of individuals with penicillin allergy in both genders.

The results show that none of the individuals infected with EBV-DNA had received any corticosteroid therapy nor did they have any immune diseases. Epstein-Barr virus DNA was found in 1/1 (100.0%) of female patients who had received chemotherapy compared to 5/11 (45.5%) in individuals who received no therapies.

5.1.7 EBV infection in relation to UK-PVL

The results for EBV infection in relation to PVL are summarised in Table 5.4. These crude results indicate that in this UK sample, people who have PVL are 7.33 times more likely to be infected with EBV than normal controls. Although the adjusted results (adjusted for age, ethnicity, smoking, Paan use and alcohol drinking), indicate that in this UK sample, people who

are PVL are 173.34 times more likely to be infected with EBV than normal controls. However, it should be noted that the sample size is small and the very wide C.I indicated a high level of uncertainity from this result.

Table 5.5 Detection of Epstein-Barr virus versus demographics and risk factors for oral squamous cell carcinoma of UK patients with oral epithelial dysplasia

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Gender						
Female	22/22(100.0)	0/22(0.0)				
Male	22/23(95.7)	1/23(4.3)				
Age (years)						
20-30	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
0-40	2/2(100.0)	0/2(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)
40-50	3/3(100.0)	0/3(0.0)	1/1(100.0)	0/1(0.0)	2/2(100.0)	0/2(0.0)
50-60	16/16(100.0)	0/16(0.0)	6/6(100.0)	0/6(0.0)	10/10(100.0)	0/10(0.0)
60-70	14/14(100.00	0/14(0.0)	7/7(100.0)	0/7(0.0)	7/7(100.0)	0/7(0.0)
70-80	9/9(100.0)	0/9(0.0)	7/7(100.0)	0/7(0.0)	7/7(100.00	0/7(0.0)
Ethnicity						
Caucasian	30/30(100.0)	0/30(0.0)	14/14(100.0)	0/14(0.0)	16/16(100.0)	0/16(0.0)
SEA*	5/6(83.3)	1/6(16.7)	4/4(100.0)	0/4(0.0)	11/12(91.7)	1/12(8.3)
N/A**	9/9(100.0)	0/9(0.0)	9/9(100.0)	0/9(0.0)	5/5(100.0)	0/5(0.0)
Tobacco						
None	23/24(95.8)	1/24(4.2)	15/15(100.0)	0/15(0.0)	8/9(88.9)	1/9(11.1)
Ex-smoker	3/3(100.0)	0/3(0.0)	-	-	3/3(100.0)	0/3(0.0)
Current	18/18(100.0)	0/18(0.0)	7/7(100.0)	0/7(0.0)	11/11(100.0)	0/11(0.0)
Smoking						
None	23/24(95.8)	1/24(4.2)	15/15(100.0)	0/15(0.0)	8/9(88.9)	1/9(11.1)
10-15/day	12/12(100.0)	0/12(0.0)	4/4(100.0)	0/4(0.0)	8/8(100.0)	0/8(0.0)
>20/day	9/9(100.0)	0/9(0.0)	3/3(100.0)	0/3(0.0)	6/6(100.0)	0/6(0.0)
Paan						
None	40/40(400.0)	0/40(0.0)	19/19(100.0)	0/19(0.0)	21/21(100.0)	0/21(0.0)
Current	4/5(80.0)	1/5(20.0)	3/3(100.0)	0/3(0.0)	1/2(50.0)	1/2(50.0)
Alcohol						
None	22/23(95.7)	1/23(4.3)	11/11(100.0)	0/11(0.0)	11/12(91.7)	1/12(8.3)
< 5 u/w ***	4/4(100.0)	0/4(0.0)	3/3(100.0)	0/3(0.0)	1/1(100.0)	0/1(0.0)
5-20 u/ w	3/3(100.0)	0/3(0.0)	1/1(100.0)	0/1(0.0)	2/2(100.0)	0/2(0.0)
> 20 u/w	12/12(100.0)	0/12(0.0)	6/6(100.0)	0/6(0.0)	6/6(100.0)	0/6(0.0)
N/A**	3/3(100.0)	0/3(0.0)	1/1(100.0)	0/1(0.0)	2/2(100.0)	0/2(0.0)
Total	**44/45(97.8)**	**1/45(2.2)**	**22/22(100.0)**	**0/22(0.0)**	**22/23(95.7)**	**1/23(4.3)**

*SEA=South East Asian
**N/A= Data not available
*** u/w = units per week

Table 5.6 Cross tabulation for Epstien-Barr virus in UK oral epithelial dysplasia samples according to biopsy site, relevant medical note, corticosteroids, immune status and therapies

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Biopsy site						
Buccal mucosa	7/8(87.5)	1/8(12.5)	3/3(100.0)	0/3(0.0)	4/5(80.0)	1/5(20.0)
Lt. of tongue*	12/12(100.0)	0/12(0.0)	6/6(100.0)	0/6(0.0)	6/6(100.0)	0/6(0.0)
Floor of mouth	3/3(100.0)	0/3(0.0)	2/2(100.0)	0/2(0.0)	1/1(100.0)	0/1(0.0)
Hard palate	2/2(100.0)	0/2(0.0)	-	-	2/2(100.0)	0/2(0.0)
Soft palate	2/2(100.0)	0/2(0.0)	-	-	2/2(100.0)	0/2(0.0)
Ant. 2/3 of tongue**	4/4(100.0)	0/4(0.0)	4/4(100.0)	0/4(0.0)	-	-
Lip	2/2(100.0)	0/2(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)
Commisure	9/9(100.0)	0/9(0.0)	3/3(100.0)	0/3(0.00	6/6(100.0)	0/6(0.0)
Retromolar	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Salivary	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Attached gingiva	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Relevant medical note						
None	20/20(100.0)	0/20(0.0)	11/11(100.0)	0/11(0.0)	9/9(100.0)	0/9(0.0)
SCC	7/7(100.0)	0/7(0.0)	2/2(100.0)	0/2(0.0)	5/5(100.0)	0/5(0.0)
Candidal infection	5/5(100.0)	0/5(0.0)	2/2(100.0)	0/2(0.0)	3/3(100.0)	0/3(0.0)
Submucous fibrosis	3/4(75.0)	1/4(25.0)	2/2(100.0)	0/2(0.0)	1/2(50.0)	1/2(50.00
HRT****	5/5(100.0)	0/5(0.0)	5/5(100.0)	0/5(0.0)	-	-
Arthritis	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Asthma	2/2(100.0)	0/2(0.0)	-	-	2/2(100.0)	0/2(0.0)
Diabetes mellitus	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Corticosteroids						
None	44/45(97.8)	1/45(2.2)	22/22(100.0)	0/22(0.0)	22/23(95.7)	1/23(4.3)
Immune status						
None	42/43(97.7)	1/43(2.3)	21/21(100.0)	0/21(0.0)	21/22(95.5)	1/22(4.5)
Systemic	2/2(100.0)	0/2(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)
Therapies						
None	40/41(97.6)	1/41(2.4)	18/18(100.0)	0/18(0.0)	22/23(95.7)	1/23(4.3)
Chemotherapy	4/4(100.0)	0/4(0.0)	4/4(100.0)	0/4(0.0)	-	-
Total	**44/45(97.8)**	**1/45(2.2)**	**22/22(100.0)**	**0/22(0.0)**	**22/23(95.7)**	**1/23(4.3)**

* Lt. of tongue= lateral border of the tongue
** Ant. 2/3 of tongue= anterior 2/3 of tongue
***SCC=Second primary squamous cell carcinoma
****HRT=Hormone replacement therapy

Table 5.7 Epstein-Barr virus infection in relation to UK-oral epithelial dysplasia samples

UK	Gender				Crude Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)
OED						
	All		OED			
			-	+		
		EBV -	44	44	0.17(0.02,1.44)	N/C¶¶
		EBV +	6	1		
			OED			
			-	+		
	F	EBV -	26	22	N/C¶	N/C¶
		EBV +	3	0		
			OED			
			-	+		
	M	EBV -	18	22	0.27(0.03, 2.85)	N/C¶¶
		EBV +	3	1		

¶ N/C= OR could not be calculated because none of the OED was EBV infected
¶¶ N/C= Adjusted OR could not be calculated because none of the OED was EBV infected in at least 1 subgroup

5.2 Epstein-Barr in oral epithelial dysplasia - UK

The frequency of Epstein-Barr DNA virus in OED was summarised in Table 5.1. An overall EBV-DNA positive proportion of 1/45 (2.2%) was detected in OED-UK samples. Demographic characteristics and risk factors for OED in the UK patients are summarised in Table 5.2.

5.2.1 Epstein-Barr virus demographic Characteristics

Epstein-Barr virus was only detected in one sample. This person was a South East Asian male aged 20-30 who was a non-smoker, non-drinker and a current user of Paan. He had a submucous fibrosis and the site of the lesion was the buccal mucosa. This patient had no history of any immune disease nor had received any therapy.

5.2.7 EBV infection in relation to UK-OED

The results based on the logistic regression analysis (the odds ratios (OR) and their corresponding 95% confidence intervals) for EBV in OED are summarised in Table 5.7. The crude results revealed an overall OR of 0.17; 95% CI= 0.02-1.44 (p=0.104). When stratified according gender and risk factors (age, ethnicity, smoking, alcohol and Paan), the crude results of OR in females could not be calculated because none of the OED was EBV infected. The OR of the males group of 0.27; 95% CI= 0.03-2.85, (p=0.278), and the adjusted OR could not be calculated because none of the OED was EBV infected in at least 1 subgroup.

Table 5.8 Demographics details of UK normal patients

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Gender						
Female	26/29(89.7)	3/29(10.3)				
Male	18/21(85.7)	3/21(14.3)				
Age (years)						
<20	4/6(66.7)	2/6(33.3)	3/5(60.0)	2/5(40.0)	1/1(100.0)	0/1(0.0)
20-30	10/11(90.9)	1/11(9.1)	6/6(100.0)	0/6(0.0)	4/5(80.0)	1/5(20.0)
30-40	7/8(87.5)	1/8(12.5)	4/4(100.0)	0/4(0.0)	3/4(75.0)	1/4(25.0)
40-50	6/6(100.0)	0/6(0.0)	4/4(100.0)	0/4(0.0)	2/2(100.0)	0/2(0.0)
50-60	7/8(87.5)	1/8(12.5)	3/3(100.0)	0/3(0.0)	4/5(80.0)	1/5(20.0)
60-70	3/4(75.0)	1/4(25.0)	3/4(75.0)	1/4(25.0)	-	-
70-80	4/4(100.0)	0/4(0.0)	2/2(100.0)	0/2(0.0)	2/2(100.0)	0/2(0.0)
>80	3/3(100.0)	0/3(0.0)	1/1(100.0)	0/1(0.0)	2/2(100.0)	0/2(0.0)
Ethnicity						
Caucasian	33/37(89.2)	4/37(10.8)	22/23(95.7)	1/23(4.3)	11/14(78.6)	3/14(21.4)
SEA*	9/10(90.0)	1/10(10.0)	4/5(80.0)	1/5(20.0)	5/5(100.0)	0/5(0.0)
African	0/1(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	-	-
Arab	2/2(100.0)	0/2(0.0)	-	-	2/2(100.0)	0/2(0.0)
Tobacco						
None	43/49(87.8)	6/49(12.2)	26/29(89.7)	3/29(10.3)	17/20(85.0)	3/20(15.0)
Ex-smoker	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Smoking						
None	43/49(87.8)	6/49(12.2)	26/29(89.7)	3/29(10.3)	17/20(85.0)	3/20(15.0)
10-15/day	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Paan						
None	42/48(87.5)	6/48(12.5)	26/29(89.7)	3/29(10.3)	16/19(84.2)	3/19(15.8)
Ex-user	2/2(100.0)	0/2(0.0)	-	-	2/2(100.0)	0/2(0.0)
Alcohol						
None	40/46(87.0)	6/46(13.0)	23/26(88.5)	3/26(11.5)	17/20(85.0)	3/20(15.0)
5-20 u/w	4/4(100.0)	0/4(0.0)	3/3(100.0)	0/3(0.0)	1/1(100.0)	0/1(0.0)
Total	**44/50(88.0)**	**6/50(12.0)**	**26/29(89.7)**	**3/29(10.3)**	**18/21(85.7)**	**3/21(14.3)**

*SEA= South East Asian

Table 5.9 Cross tabulation for Epstein-Barr virus in normal oral samples according to biopsy site, relevant medical note, corticosteroids, immune status and therapies

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Biopsy site						
Buccal mucosa	10/11(90.9)	1/11(9.1)	7/7(100.0)	0/7(0.0)	3/4(75.0)	1/4(25.0)
Lt. of tongue*	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Attached gingiva	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Soft palate	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Ant.2/3 of tongue**	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Retromolar	2/2(100.0)	0/2(0.0)	-	-	2/2(100.0)	0/2(0.0)
Floor of mouth	10/12(83.3)	2/12(16.7)	6/7(85.7)	1/7(14.3)	4/5(80.0)	1/5(20.0)
Hard palate	3/4(75.0)	1/4(25.0)	2/3(66.7)	1/3(33.3)	1/1(100.0)	0/1(0.0)
Lip	15/17(88.2)	2/17(11.8)	9/10(90.0)	1/10(10.0)	6/7(85.7)	1/7(14.3)
Relevant medical note						
None	43/47(91.5)	4/47(8.5)	25/27(92.6)	2/27(7.4)	18/20(90.0)	2/20(10.0)
Asthma	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
HRT***	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Penicillin allergy	0/1(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	-	-
Corticosteroids						
None	43/49(87.8)	6/49(12.2)	25/28(89.3)	3/28(10.7)	18/21(85.7)	3/21(14.3)
systematic	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Immune status						
None	44/50(88.0)	6/50(12.0)	26/29(89.7)	3/29(10.3)	18/21(85.7)	3/21(14.3)
Therapies						
None	44/50(88.0)	6/50(12.0)	26/29(89.7)	3/29(10.3)	18/21(85.7)	3/21(14.3)
Total	**44/50(88.0)**	**6/50(12.0)**	**26/29(89.7)**	**3/29(10.3)**	**18/21(85.7)**	**3/21(14.3)**

*Lt. of tongue= lateral border of the tongue
**Ant2/3 of tongue= anterior 2/3 of the tongue
***HRT= Hormone replacement therapy

5.3 Epstein-Barr virus in normal oral mucosa-UK

The frequency of Epstein-Barr virus DNA among oral lesions is summarised in Table 5.1. An overall EBV-DNA positive rate of 6/50 (12.0%) was detected in normal control oral mucosa samples. Demographic characteristics and risk factors for normal oral mucosa in the UK patients are summarised in Table 5.

5.3.1 Gender

Epstein-Barr DNA was detected in 3/29 (10.3%) of females compared with 3/21 (14.3%) in males.

5.3.2 Age groups

EBV-DNA was examined in different age groups. Epstein-Barr DNA was detected in 2/5 (40.0%) of females <20 year age group and in 1/4 (25.0%) of females 60-70 year age group, EBV was found in male group in 1/5 (20.0%) 20-30 year age group, 1/4 (25.0%) in 30-40 year age group and 1/5 (20.0%) of 50-60 year age group.

5.3.3 Ethnic origins

Epstein-Barr DNA was found in 4/37(10.8%) of Caucasians, 1/10(10.0%) and 1/1(100.0%) in South East Asians and Africans respectively.

5.3.4 Association with tobacco, alcohol and Paan

The association between EBV and other major risk factors for normal-control oral mucosa samples (e.g. tobacco smoking, frequency of smoking,

Paan use and alcohol intake) was examined. None of the EBV positive patients were smokers, Paan-users or alcohol consumers.

5.3.5 Site of normal-control oral mucosa samples

Cross tabulation for EBV-DNA frequency and other characteristics are summarised in Table 5. With regard to EBV-DNA and biopsy site, EBV-DNA was detected in the buccal mucosa, floor of the mouth and lip of males and in the lateral border of the tongue, hard palate and lip of females.

5.3.6 Relevant medical history

Epstein-Barr virus DNA was positive in both genders with no relevant medical history and in one asthmatic male and one female who is allergic to penicillin. None of the patients had received any corticosteroid therapy or other treatments and had no immune problems.

Chapter 6

Cytomegalovirus- Results

Table 6.1 Frequency of cytomegalovirus in examined oral mucosa

Country	Lesion	Gender	-ve No. (%)	+ve No. (%)
UK				
	PVL	All	10/12 (83.3)	2/12 (16.7)
		F	5/6 (83.3)	1/6 (16.7)
		M	5/6 (83.3)	1/6 (16.7)
	OED	All	38/45(84.4)	7/45 (15.6)
		F	19/22 (86.4)	3/22 (13.6)
		M	19/23 (82.6)	4/23 (17.4)
	Normal control	All	49/50(98.0)	1/50(2.0)
		F	28/29(96.9)	1/29(3.4)
		M	-	-

PVL = Proliferative verrucous leukoplakia
OED= Oral epithelial dysplasia

Table 6.2 Detection of cytomegalovirus versus demographics and risk factors for oral squamous cell carcinoma of UK pateints with proliferative verrucous leukoplakia

Characteristic		-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Gender							
	Female	5/6(83.3)	1/6(16.7)				
	Male	5/6(83.3)	1/6(16.7)				
Age (years)							
	40-50	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
	50-60	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
	60-70	2/3(66.7)	1/3(33.3)	1/2(50.0)	1/2(50.0)	1/1(100.0)	0/1(0.0)
	70-80	5/6(83.3)	1/6(16.7)	3/3(100.0)	0/3(0.0)	2/3(66.7)	1/3(33.3)
	>80	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Ethnicity							
	Caucasian	3/5(60.0)	2/5(40.0)	3/4(75.0)	1/4(25.0)	0/1(0.0)	1/1(100.0)
	N/A*	7/7(100.0)	0/7(0.0)	2/2(100.0)	0/2(0.0)	5/5(100.0)	0/5(0.0)
Tobacco							
	None	9/10(90.0)	1/10(10.0)	5/6(83.3)	1/6(16.7)	4/4(100.0)	0/4(0.0)
	Current	1/2(50.0)	1/2(50.0)	-	-	1/2(50.0)	1/2(50.0)
Smoking							
	None	9/10(90.0)	1/10(10.0)	5/6(83.3)	1/6(16.7)	4/4(100.0)	0/4(0.0)
	>20/day	1/2(50.0)	1/2(50.0)	-	-	1/2(50.0)	1/2(50.0)
Paan							
	None	9/11(81.8)	2/11(18.2)	5/6(83.3)	1/6(16.7)	4/5(80.0)	1/5(20.0)
	Current	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Alcohol							
	None	10/11(90.0)	1/11(9.1)	5/6(83.3)	1/6(16.7)	5/5(100.0)	0/5(0.0)
	>20 u/w**	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Total		**10/12(83.3)**	**2/12(16.7)**	**5/6(83.3)**	**1/6(16.7)**	**5/6(83.3)**	**1/6(16.7)**

*N/A= Data not available
** u/w = units per week

Table 6.3 Cross tabulation for cytomegalovirus in UK proliferative verrucous leukoplakia samples according to biopsy site, relevant medical note, corticosteroids, immune status and therapies

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Biopsy site						
Buccal mucosa	3/4(75.0)	1/4(25.0)	3/4(75.0)	1/4(25.0)	-	-
Lt. of tongue*	2/2(100.0)	0/2(0.0)	-	-	2/2(100.0)	0/2(0.0)
Floor of mouth	1/2(50.0)	1/2(50.0)	-	-	1/2(50.0)	1/2(50.0)
Hard palate	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Ant. 2/3 of tongue**	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Lip	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Retromolar	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Relevant medical note						
None	3/3(100.0)	0/3(0.0)	2/2(100.0)	0/2(0.0)	1/1(100.0)	0/1(0.0)
SCC***	5/6(83.3)	1/6(16.7)	2/3(66.7)	1/3(33.3)	3/3(100.0)	0/3(0.0)
Candidal infection	0/1(0.0)	1/1(100.0)	-	-	0/1(0.0)	1/1(100.0)
Penicillin allergy	2/2(100.0)	0/2(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)
Corticosteroids						
None	10/12(83.3)	2/12(16.7)	5/6(83.3)	1/6(16.7)	5/6(83.3)	1/6(16.7)
Immune status						
None	10/12(83.3)	2/12(16.7)	5/6(83.3)	1/6(16.7)	5/6(83.3)	1/6(16.7)
Therapies						
None	10/11(90.9)	1/11(9.1)	5/5(100.0)	0/5(0.0)	5/6(83.3)	1/6(16.7)
Chemotherapy	0/1(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	-	-
Total	**10/12(83.3)**	**2/12(16.7)**	**5/6(83.3)**	**1/6(16.7)**	**5/6(83.3)**	**1/6(16.7)**

* Lt. of tongue= lateral border of the tongue
** Ant. 2/3 of tongue= anterior 2/3 of tongue
***SCC= Second primary Squamous Cell Carcinoma

Table 6.4 Cytomegalovirus infection in relation to UK-proliferative verrucous leukoplakia samples

UK	Gender				Odds Ratio (95% CI)
PVL					
	All		PVL -	PVL +	
		CMV -	49	10	9.80(0.81,118.79)
		CMV +	1	2	
	F		PVL -	PVL +	
		CMV -	28	5	5.60(0.30, 104.94)
		CMV +	1	1	
	M		PVL -	PVL +	
		CMV -	21	5	N/C
		CMV +	0	1	

N/C= OR could not be calculated because none of the normal controls was CMV+ve in the males.

6.1 Cytomegalovirus in proliferative verrucous leukoplakia - UK

The frequency of cytomegalovirus DNA among oral lesions is summarised in Table 6.1. An overall CMV-DNA positive proportion of 2/12 (16.7%) was detected in PVL-UK patients. Demographic characteristics and risk factors for PVL in the UK patients are summarised in Table 6.2.

6.1.1 Gender

Cytomegalovirus-DNA was detected in 1/6 (16.7%) of each gender.

6.1.2 Age groups

When CMV-DNA was examined in different age groups, CMV-DNA was detected in 1/3 (33.3%) of 60-70 year age group and in 1/6 (16.7%) of the 70-80 year age group. When stratified according to gender and age, CMV-DNA was found in 1/2 (50.0%) of female aged 60-70 years and in 1/3 (33.3%) of males aged 70-80 years.

6.1.3 Ethnic origins

Cytomegalovirus-DNA was detected in 2/5 (40.0%) of Caucasians. When stratified according to gender and ethnicity, CMV-DNA was found in 1/4 (25.0%) of females and in 1/1 (100.0%) of males.

6.1.4 Association with tobacco, alcohol and Paan

The association between CMV and other major risk factors for PVL (e.g. tobacco smoking, frequency of smoking, Paan use and alcohol intake) was examined. Cytomegalovirus-DNA was found in 1/10 (10.0%) of the non-smoking group compared with 1/2 (50.0%) of individuals smoking > 20 /day. When stratified according to gender and smoking, none of the females in this

group were current smokers. CMV-DNA was found in 1/2 (50.0%) of male current smokers.

When the use of Paan was examined, CMV-DNA was detected in 2/11 (18.2%) of non-Paan users. When stratified according to gender and use of Paan, CMV-DNA was found in 1/6 (16.7%) of females compared with 1/5 (20.0%) in males.

The proportion of CMV-DNA was detected in 1/11 (9.1%) of non-alcohol drinkers compared with 1/1 (100.0%) in individuals currently consuming >20 units per week. When stratified according to gender and alcohol intake, the results show that none of the females in this group were current alcohol consumers compared with 1/1 (100.0%) of males consuming >20 units per week.

6.1.5 Site of proliferative verrucous leukoplakia samples

Cross tabulation for CMV-DNA frequency and other characteristics are summarised in Table 6.3. Cytomegalovirus-DNA was detected in 1/4 (25.0%) of the buccal mucosa samples of females and in 1/2 (50.0%) of the floor of mouth samples in males.

6.1.6 Relevant medical history

Cytomegalovirus-DNA was detected in 1/6 (16.7%) of patients with second primary OSCC and in 1/1 (100.0%) of patients with candidal infection. When stratified according to gender and medical history, CMV-DNA was found in 1/3 (33.3%) of females with second primary and in 1/1 (100.0%) of males with candidal infection.

The results show that none of the individuals were infected with CMV-DNA had received any corticosteroid therapy nor suffered from any known immune disease. Cytomegalovirus-DNA was found in 1/1 (100.0%) of patients who received chemotherapy compared with 1/11 (9.1%) of patients who had received no therapy.

6.1.7 CMV infection in relation to UK-PVL

The results based on the logistic regression analysis (the odds ratios (OR) and their corresponding 95% confidence intervals) for CMV in PVL are summarised in Table 6.4. The crude results indicate that in this UK sample, people who have PVL are 9.80 times more likely to be infected with CMV than normal controls. Adjusted OR could not be calculated because none of the PVL was CMV infected in at least one subgroup. It should be noted that the sample size is relatively small and the very wide C.I indicated a high level of uncertainity for this result.

Table 6.5 Detection of cytomegalovirus versus demographics and risk factors for oral squamous cell carcinoma of UK patients with oral epithelial dysplasia

Characteristic		-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Gender							
	Female	19/22(86.4)	3/22(13.6)				
	Male	19/23(82.6)	4/23(17.4)				
Age (years)							
	20-30	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
	30-40	2/2(100.0)	0/2(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)
	40-50	2/3(66.7)	1/3(33.3)	0/1(0.0)	1/1(100.0)	2/2(100.0)	0/2(0.0)
	50-60	15/16(93.8)	1/16(6.3)	6/6(100.0)	0/6(0.0)	9/10(90.0)	1/10(10.0)
	60-70	11/14(78.6)	3/14(21.4)	6/7(85.7)	1/7(14.3)	5/7(71.4)	2/7(28.6)
	70-80	7/9(77.8)	2/9(22.2)	6/7(85.7)	1/7(14.3)	1/2(50.0)	1/2(50.0)
Ethnicity							
	Caucasian	25/30(83.3)	5/30(16.7)	11/14(78.6)	3/14(21.4)	14/16(87.5)	2/16(12.5)
	SEA*	5/6(83.3)	1/6(16.7)	4/4(100.0)	0/4(0.0)	1/2(50.0)	1/2(50.0)
	N/A**	8/9(88.9)	1/9(11.1)	4/4(100.0)	0/4(0.0)	4/5(80.0)	1/5(20.0)
Tobacco							
	None	20/24(83.3)	4/24(16.7)	14/15(93.3)	1/15(6.7)	6/9(66.7)	3/9(33.3)
	Ex-smoker	2/3(66.7)	1/3(33.3)	-	-	2/3(66.7)	1/3(33.3)
	Current	16/18(88.9)	2/18(11.1)	5/7(71.4)	2/7(28.6)	11/11(100.0)	0/11(0.0)
Smoking							
	None	20/24(83.3)	4/24(16.7)	14/15(93.3)	1/15(6.7)	6/9(66.7)	3/9(33.3)
	10-15/day	10/12(83.3)	2/12(16.7)	2/4(50.0)	2/4(50.0)	8/8(100.0)	0/8(0.0)
	>20/day	8/9(88.9)	1/9(11.1)	3/3(100.0)	0/3(0.0)	5/6(83.3)	1/6(16.7)
Paan							
	None	34/40(85.0)	6/40(15.0)	16/19(84.2)	3/19(15.8)	18/21(85.7)	3/21(14.3)
	Current	4/5(80.0)	1/5(20.0)	3/3(0.0)	0/3(0.0)	1/2(50.0)	1/2(50.0)
Alcohol							
	None	20/23(87.0)	3/23(13.0)	11/11(100.0)	0/11(0.0)	9/12(75.0)	3/12(25.0)
	<5 u/w***	4/4(100.0)	0/4(0.0)	3/3(100.0)	0/3(0.0)	1/1(100.0)	0/1(0.0)
	5-20 u/w	2/3(66.7)	1/3(33.3)	0/1(0.0)	1/1(100.0)	2/2(100.0)	0/2(0.0)
	> 20 u/w	9/12(75.0)	3/12(25.0)	4/6(66.7)	2/6(33.3)	5/6(83.3)	1/6(16.7)
	N/A	3/3(100.0)	0/3(0.0)	1/1(100.0)	0/1(0.0)	2/2(100.0)	0/2(0.0)
Total		**38/45(84.4)**	**7/45(15.6)**	**19/22(86.4)**	**3/22(13.6)**	**19/23(82.6)**	**4/23(17.4)**

*SEA=South East Asian
**N/A= Data not available
*** u/w = units per week

Table 6.6 Cross tabulation for cytomegalovirus in UK-oral epithelial dysplasia samples according to biopsy site, relevant medical note, corticosteroids, immune status, and therapies

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Biopsy site						
Buccal mucosa	6/8(75.0)	2/8(25.0)	3/3(100.0)	0/3(0.0)	3/5(60.0)	2/5(40.0)
Lt. of tongue*	9/12(75.0)	3/12(25.0)	4/6(66.7)	2/6(33.3)	5/6(83.3)	1/6(16.7)
Floor of mouth	3/3(100.0)	0/3(0.0)	2/2(100.0)	0/2(0.0)	1/1(100.0)	0/1(0.0)
Hard palate	1/2(50.0)	1/2(50.0)	-	-	1/2(50.0)	1/2(50.0)
Soft palate	2/2(100.0)	0/2(0.0)	-	-	2/2(100.0)	0/2(0.0)
Ant. 2/3 of tongue**	3/4(75.0)	1/4(25.0)	3/4(75.0)	1/4(25.0)	-	-
Lip	2/2(100.0)	0/2(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)
Commisure	9/9(100.0)	0/9(0.0)	3/3(100.0)	0/3(0.0)	6/6(100.0)	0/6(0.0)
Retromolar	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Salivary	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Attached gingiva	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Relevant medical note						
None	18/20(90.0)	2/20(10.0)	9/11(81.8)	2/11(18.2)	9/9(100.0)	0/9(0.0)
SCC***	5/7(71.4)	2/7(28.6)	2/2(100.0)	0/2(0.0)	3/5(60.0)	2/5(40.0)
Candidal infection	4/5(80.0)	1/5(20.0)	2/2(100.0)	0/2(0.0)	2/3(66.7)	1/3(33.3)
Submucous fibrosis	3/4(75.0)	1/4(25.0)	2/2(100.0)	0/2(0.0)	1/2(50.0)	1/2(50.0)
HRT****	4/5(80.0)	1/5(20.0)	4/5(80.0)	1/5(20.0)	-	-
Arthritis	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Asthma	2/2(100.0)	0/2(0.0)	-	-	2/2(100.0)	0/2(0.0)
Diabetic	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Corticosteroids						
None	38/45(84.4)	7/45(15.6)	19/22(86.4)	3/22(13.6)	19/23(82.6)	4/23(17.4)
Immune status						
None	36/43(83.7)	7/43(16.3)	18/21(85.7)	3/21(14.3)	18/22(81.8)	4/22(18.2)
Systemic	2/2(100.0)	0/2(0.0)	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)
Therapies						
None	35/41(85.4)	6/41(14.6)	16/18(88.9)	2/18(11.1)	19/23(82.6)	4/23(17.4)
Chemotherapy	3/4(75.0)	1/4(25.0)	3/4(75.0)	1/4(25.0)	-	-
Total	**38/45(84.4)**	**7/45(15.6)**	**19/22(86.4)**	**3/22(13.6)**	**19/23(82.6)**	**4/23(17.4)**

* Lt. of tongue= lateral border of the tongue
** Ant. 2/3 of tongue= anterior 2/3 of tongue
***SCC= second primary Squamous Cell Carcinoma
****HRT=Hormone replacement therapy

Table 6.7 Cytomegalovirus infection in relation to UK-oral epithelial dysplasia samples

UK	Gender				Odds Ratio (95% CI)
OED					
	All		OED		
			-	+	
		CMV -	49	38	9.03(1.06, 76.54)
		CMV +	1	7	
			OED		
			-	+	
	F	CMV -	28	19	4.42(0.43, 45.76)
		CMV +	1	3	
			OED		
			-	+	
	M	CMV -	21	19	N/C
		CMV +	0	4	

N/C= OR could not be calculated because none of the normal control were CMV+ve in the males.

6.2 Cytomegalovirus in oral epithelial dysplasia of patients from UK

The frequency of cytomegalovirus DNA among oral lesions is summarised in Table 6.1. An overall CMV-DNA positive proportion of 7/45 (15.6%) was detected in OED-UK patients. Demographic characteristics and risk factors for OED in the UK patients are summarised in Table 6.5.

6.2.1 Gender

Cytomegalovirus-DNA was detected in 3/22 (13.6%) of females compared with 4/23 (17.4%) in males.

6.2.2 Age groups

When CMV-DNA was examined in different age groups, CMV-DNA was found in 1/3 (33.3%) of the 40-50 year age group, in 1/16 (6.3%) of the 50-60 year age group, in 3/14 (21.4%) of the 60-70 year age group and in 2/9 (22.2%) of the 70-80 year age group. When stratified according to age and gender, CMV-DNA was found in 1/1 (100.0%) in the female 40-50 year age group and in 1/7 (14.3%) of both 60-70 and 70-80 year age female groups. Cytomegalovirus-DNA was detected in 2/7 (28.6%) of the 60-70 year age group compared with 1/2 (50.0%) of the 70-80 year age male group.

6.2.3 Ethnic origins

Cytomegalovirus-DNA was detected in 5/30 (16.7%) of Caucasians compared with 1/6 (16.7%) in South East Asians. When stratified according to gender and ethnicity, CMV-DNA was found in 3/14 (21.4%) of Caucasian females compared with 2/16 (12.5%) of Caucasian males. However, CMV-DNA was found in 1/2 (50.0%) of South East Asians. CMV-DNA was detected in 1/9 (11.1%) in individuals with no recorded ethnicity.

6.2.4 Association with tobacco, alcohol and Paan

The association between CMV and other major risk factors for OED (e.g. tobacco smoking, frequency of smoking, Paan use and alcohol intake) was examined. Cytomegalovirus-DNA was found in 4/24 (16.7%) of non-smokers compared with 1/3 (33.3%) in the ex-smoking group and in 2/18 (11.1%) of current smokers. Among the smoking group, CMV-DNA was detected in 2/12 (16.7%) of individuals smoking 10-15 /day, and in 1/9 (11.1%) of individuals smoking >20/day. When stratified according to gender and smoking, CMV-DNA was detected in 2/4 (50.0%) of females smoking 10-15/day and in 1/15 (6.7%) of non-smokers females. CMV was found in 1/6 (16.7%) of males smoking >20/day and in 3/9 (33.3%) of non-smoker males.

When the use of Paan was examined, CMV-DNA was found in 6/40 (15.0%) of non-Paan users compared with 4/5 (80.0%) of current Paan users. When stratified according to gender and use of Paan, CMV-DNA was detected in 3/19 (15.8%) of females compared with 3/21 (14.3%) of males.

Cytomegalovirus-DNA was found in 3/23 (13.0%) of non-alcohol drinkers compared with 1/3 (33.3%) of individuals currently consuming 5-20 units per week and in 3/12 (25.0%) of individuals currently consuming >20 units per week. When stratified according to gender and alcohol intake, the results show that CMV-DNA was found in 1/1 (100.0%) of females consuming 5-20 units per week compared with 2/6 (33.3%) of females consuming > 20 units per week. Among the males group, CMV-DNA was detected in 3/12 (25.0%) of non-alcohol drinkers compared with 1/6 (16.7%) of males consuming > 20 units per week.

6.2.5 Site of oral epithelial dysplasia samples

Cross tabulation for CMV-DNA frequency and other characteristics are summarised in Table 6.6. Cytomegalovirus-DNA was detcted in the buccal mucosa, lateral border of the tongue, hard palate and anterior two thirds of tongue. When stratified according to gender and biopsy site, no difference was readily apparent as regard the biopsy site and CMV infection.

6.2.6 Relevant medical history

Cytomegalovirus-DNA was detected in 2/20 (10.0%) of patients with no relevant medical history, in 2/7 (28.6%) of patients with second primary OSCC, in 1/5 (20.0%) of patients with candidal infection and in 1/4 (25.0%) of patients with submucous fibrosis. When stratified according to gender and relevant medical history, CMV was found in 1/5 (20.0%) of females received HRT compared with 2/11 (18.2%) of females with no relevant medical history. CMV-DNA was detected in 2/5 (40.0%) of male patients with second-primary OSCC, in 1/3 (33.3%) of male patients with candidal infection and in 1/2 (50.0%) of male patients with submucous fibrosis.

The results show that none of the individuals infected with CMV-DNA had received any corticosteroids nor had any known immune disease. Cytomegalovirus-DNA was detected in 1/4 (25.0%) of individuals who had received chemotherapy compared with 6/41 (14.6%) of individuals who received no therapy. When stratified according to gender and treatment, CMV-DNA was found in 1/4 (25.0%) of female patients who had received chemotherapy. Cytomegalovirus-DNA was found in 2/18 (11.1%) of females who received no therapy compared with 4/23 (17.4%) of males within the same group.

6.2.7 CMV infection in relation to UK-OED

The results based on the logistic regression analysis (the odds ratios (OR) and their corresponding 95% confidence intervals) for CMV in OED are summarised in Table 6.7. The crude results indicate that in this UK sample, people who have OED are 9.03 times more likely to be infected with CMV than normal controls. Adjusted OR could not be calculated because none of the OED was CMV infected in at least one subgroup. It should be noted that the sample size is relatively small and the very wide C.I indicated a high level of uncertainity for this result.

Table 6.8 Demographics details of UK normal pateints

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Gender						
Female	28/29(96.6)	1/29(3.4)				
Male	21/21(100.0)	0/21(0.0)				
Age (years)						
<20	5/6(83.3)	1/6(16.7)	4/5(80.0)	1/5(20.0)	1/1(100.0)	0/1(0.0)
20-30	11/11(100.0)	0/11(0.0)	6/6(100.0)	0/6(0.0)	5/5(100.0)	0/5(0.0)
30-40	8/8(100.0)	0/8(0.0)	4/4(100.0)	0/4(0.0)	4/4(100.0)	0/4(0.0)
40-50	6/6(100.0)	0/6(0.0)	4/4(100.0)	0/4(0.0)	2/2(100.0)	0/2(0.0)
50-60	8/8(100.0)	0/8(0.0)	3/3(100.0)	0/3(0.0)	5/5(100.0)	0/5(0.0)
60-70	4/4(100.0)	0/4(0.0)	4/4(100.0)	0/4(0.0)	-	-
70-80	4/4(100.0)	0/4(0.0)	2/2(100.0)	0/2(0.0)	2/2(100.0)	0/2(0.0)
>80	3/3(100.0)	0/3(0.0)	1/1(100.0)	0/1(0.0)	2/2(100.0)	0/2(0.0)
Ethnicity						
Caucasian	36/37(97.3)	1/37(2.7)	22/23(95.7)	1/23(4.3)	14/14(100.0)	0/14(0.0)
SEA*	10/10(100.0)	0/10(0.0)	5/5(100.0)	0/5(0.0)	5/5(100.0)	0/5(0.0)
Arab	2/2(100.0)	0/2(0.0)	-	-	2/2(100.0)	0/2(0.0)
African	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Tobacco						
None	48/49(98.0)	1/49(2.0)	28/29(96.6)	1/29(3.4)	20/20(100.0)	0/20(0.0)
Ex-smoker	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Smoking						
None	48/49(98.0)	1/49(2.0)	28/29(96.6)	1/29(3.4)	20/20(100.0)	0/20(0.0)
10-15/day	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Paan						
None	47/48(97.9)	1/48(2.1)	28/29(96.6)	1/29(3.4)	20/20(100.0)	0/20(0.0)
Ex-user	2/2(100.0)	0/2(0.0)	-	-	1/1(100.0)	0/1(0.0)
Alcohol						
None	45/46(97.8)	1/46(2.2)	25/26(96.2)	1/26(3.8)	20/20(100.0)	0/20(0.0)
5-20 u/w***	4/4(100.0)	0/4(0.0)	3/3(100.0)	0/3(0.0)	1/1(100.0)	0/1(0.0)
Total	**49/50(98.0)**	**1/50(2.0)**	**28/29(96.6)**	**1/29(3.4)**	**21/21(100.0)**	**0/21(0.0)**

*SEA=South East Asian
u/w= units per week

Table 6.9 Cross tabulation for cytomegalovirus in UK-normal control samples according to biopsy site, relevant medical note, corticosteroids, immune status and therapies

Characteristic	-ve No. (%)	+ve No. (%)	-ve Female No. (%)	+ve Female No. (%)	-ve Male No. (%)	+ve Male No. (%)
Biopsy site						
Buccal mucosa	11/11(100.0)	0/11(0.0)	7/7(100.0)	0/7(0.0)	4/4(100.0)	0/4(0.0)
Lt. of tongue*	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Floor of mouth	11/12(91.7)	1/12(8.3)	6/7(85.7)	1/7(14.3)	5/5(100.0)	0/5(0.0)
Attached gingiva	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Soft palate	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Hard palate	4/4(100.0)	0/4(0.0)	3/3(100.0)	0/3(0.0)	1/1(100.0)	0/1(0.0)
Ant. 2/3 of tongue**	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Lip	17/17(100.0)	0/17(0.0)	10/10(100.0)	0/10(0.0)	7/7(100.0)	0/7(0.0)
Retromolar	2/2(100.0)	0/2(0.0)	-	-	2/2(100.0)	0/2(0.0)
Relevant medical note						
none	46/47(97.9)	1/47(2.1)	26/27(96.3)	1/27(3.7)	20/20(100.0)	0/20(0.0)
HRT	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Asthma	1/1(100.0)	0/1(0.0)	-	-	1/1(100.0)	0/1(0.0)
Penicillin allergy	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Corticosteroids						
None	48/49(98.0)	1/49(2.0)	27/28(96.4)	1/28(3.6)	21/21(100.0)	0/21(0.0)
systematic	1/1(100.0)	0/1(0.0)	1/1(100.0)	0/1(0.0)	-	-
Immune status						
none	49/50(98.0)	1/50(2.0)	28/29(96.6)	1/29(3.4)	21/21(100.0)	0/21(0.0)
Therapies						
none	49/50(98.0)	1/50(2.0)	28/29(96.6)	1/29(3.4)	21/21(100.0)	0/21(0.0)
	49/50(98.0)	**1/50(2.0)**	**28/29(96.6)**	**1/29(3.4)**	**21/21(100.0)**	**0/21(0.0)**

* Lt. of tongue = lateral border of the tongue

6.3 Cytomegalovirus in normal samples-UK

The frequency of CMV virus DNA among oral lesions is summarised in Table 6.1. An overall CMVV-DNA positive proportion of 1/50 (2.0%) was detected in normal control oral mucosa samples. Demographic characteristics and risk factors for CMV in normal oral mucosa samples are summarised in Table 6.

6.3.1 Cytomegalovirus in normal control demographic Characteristics

Cytomegalovirus was only detected in one sample. This person was a Caucasian female aged <20 who was a non-smoker, non-drinker and had no history of using Paan. She had no relevant medical history and had received no corticosteroid or any therapy. The site of the sample was the floor of the mouth.

Chapter 7

Discussion and Conclusions

7.1 Human Papillomavirus

The present study has established that HPV was present in 79.4% and 56.7% of OSCC in persons sampled from the UK and KSA respectively. The proportions were high compared to the HPV-DNA in normal control mucosa (0.0%). Of note, the normal control samples in the present study were only obtained from UK individuals. The results of the pathological samples from both UK and KSA were compared to those of the UK normal control samples due to the lack of normal control samples from the KSA. It is accepted that it would have been better to compare the KSA samples with a group of controls drawn from the KSA, however, logistically this was difficult for the current study. Therefore, one recommendation for future research would be to recruit and analyse a group of KSA normal controls in order to either validate or refute the current results.

In a recent systematic review of published studies describing the prevalence of HPV in HNSCC biopsies world-wide (Kreimer *et al.*, 2005), the overall prevalence of HPV in OSCC was 23.5%, this being much lower than the results of the current study. However, the results of the UK samples are correlate with those from Japan whereby 74% of the examined individuals with OSCC were HPV infected (Shima *et al.*, 2000).

In the present study the high frequency of detection of HPV may reflect the use of more advanced molecular methods that can detect low viral copies within the cells (Ha and Califano, 2004).The detection of HPV in the paraffin-embedded material could have been difficult, however, the β-globin gene was detected in all examined samples, hence it is likely that HPV detection rate presently observed is real (Gravitt *et al.*, 1998; Sato *et al.*,

2001; Kreimer *et al.*, 2005). Although the methods of detection of HPV-DNA employed in the present study can detect a low number of copies (Remmerbach *et al.*, 2004; Molijn *et al.*, 2005), they do not permit the viral load within cells of the lesion, nor the expression of oncogenic E6 and E7 to be estimated (Ha *et al.*, 2002; zur Hausen, 2002; Szentirmay *et al.*, 2005). Thus, it cannot be said with definite certainty that HPV is of aetiological relevance in the present groups of HPV-infected lesions.

In the present study, HPV-16 was the only HPV type detcted in OSCC samples from both UK and KSA. Genotyping was undertaken by using a reverse line blotting technique (Jordens *et al.*, 2000) which has the ability to accurately genotype more than 24 types and to detect multiple infections, however, this technique can omit novel types of HPV (van den Brule *et al.*, 1990; Molijn *et al.*, 2005). The present results are in agreement with those of previous investigations of OSCC (Chang *et al.*, 1989; zur Hausen, 1996; Smith *et al.*, 2004; Kreimer *et al.*, 2005), indicating that it is a high-risk type of HPV that is associated with OSCC. It is known that E6 and E7 mRNA are expressed in OSCC (Hafkamp *et al.*, 2004) and thus the present results suggest that there is a viral oncogenic potential in OSCC patients both in the UK and KSA. The high detection rate of HPV-16 (known to be associated with malignancy at other mucosal surfaces) would seem to suggest that the presence of HPV-16 in the mouth might predispose to malignant transformation (Kreimer *et al.*, 2005). However, such data do not truly establish that HPV is the major cause of OSCC, as although, the OR of HPV carriage for the development of OSCC is ≈ 3.7-5.4 (independent of tobacco

and alcohol) (Syrjanen, 2005), and it is much less than the OR of HPV for cervical malignancy reaching ≈ 74 (Munoz, 2000; Garland, 2002).

Oral squamous cell carcinoma is not completely analogous to cervical cancer as HPV infection appears to be neither necessary nor sufficient for malignant progression in OSCC. The physical status of the HPV-DNA (i.e. episomal or integrated) may be of relevance in the pathogenesis of OSCC (Koskinen *et al.*, 2003). A recent study by Syrjanen in 2005, suggested that integration is an early event in HPV-induced carcinogenesis, however, in cervical malignancy integration is not essential for oncogenesis (Syrjanen, 2005). This aspect was not examined in the present study.

7.1.1 Human papillomavirus and demographic characteristics of the patients

The results of the present study indicate that the frequency of detection of HPV-DNA in OSCC in samples from the UK and KSA was 79.4% and 56.7% respectively. This difference may reflect differences in lifestyle or genetic predispositions. A study of Japanese patients found variation in the oral carriage of HPV in patients from two different geographical (and ethnically different) regions. HPV was found to be more prevalent in the Okinawa area (known to have migrants from Taiwan and Fuzuho in PR China-both reported as high HPV infection areas), than in Sapporo region (that does not have a high migrant population) (Tsuhako *et al.*, 2000). There are some geographic areas known to have a higher incidence of HNSCC than other parts, this probably reflecting local relevant habits such as chewing betel quid in Southern Asia and consumption of hot maté in South America (Castelletto *et al.*, 1994; Sewram *et al.*, 2003; Brennan and Boffetta, 2004). Such social habits are likely to underly any geographic

variations in the occurrence of OSCC rather than variations in HPV carriage rates.

Recently published studies have suggested that the prevalence of HPV in OSCC may be higher in patients from North America compared with those from Europe and Asia (Herrero *et al.*, 2003; Ha and Califano, 2004; Kreimer *et al.*, 2005). However, a review of the incidence of HPV in HNSCC in different countries (including India, France, Israel, Scotland, and North America) did not indicate that the association between HPV and HNSCC was significantly influenced by geographic locale or ethnicity (Gillison and Shah, 2001). In accordance with other studies of Europeans, the present study showed similar prevalence rates for HPV in OSCC-UK when compared with studies from Greece (94.7%) and Italy (61.5%), that both used the same methods as employed in this study (Bouda *et al.*, 2000; Giovannelli *et al.*, 2002). Such high proportions would thus be in agreement with the conclusion of the recent meta-analysis; namely that geographic locale is not a major influence of HPV carriage in OSCC (Gillison, 2004). The raised frequency of HPV infection in the UK as compared to the KSA-OSCC samples may reflect some undetectable differences in some social habits e.g. alcohol usage.

In the present study, there was no evidence of any differences in the proportion of HPV infection among Caucasian and South East Asian patients resident in the UK with OSCC. Furthermore, the current results substantiate that the use of Paan among South East Asians did not affect the proportion of HPV in the mouth, this would thus be in agreement with the frequency of HPV infections detected in a previous study of individuals with submucous fibrosis (D'Costa *et al.*, 1998).

Genders have been included in the statistical analysis of the present study in view of the fact that this research is studying HPV known to infect mostly the genital tract in females (Palefsky, 2003), and oral cancer is known to affect more males than females world-wide (Kreimer *et al.*, 2005). The results of the present study showed no evidence of differences in the prevalence of HPV infection in OSCC as regards gender which is in agreement with the findings of some (Herrero *et al.*, 2003), but not all studies, in which HPV was found to be more prevalent in males (Ringstrom *et al.*, 2002; Ritchie *et al.*, 2003). The absence of gender differences in HPV may indicate that HPV is not of aetiological significance for OSCC, as this tumour is more prevalent in males than females in the UK.

The current study found that HPV was present in patients over 40 years of age rather than in other age groups less than 40 years of age. These results were in agreement with those of other studies (Cruz *et al.*, 1996; Schwartz *et al.*, 2001), where individuals older than 60 years of age showed a high detection rate of HPV. In contrast, Hafkamp *et al.*, 2004 and Smith *et al.*, 2004 observed HPV to be more common in younger aged patients less than 40 years of age. Furthermore, a recent study by Sisk *et al.* (2000) observed that the incidence of HPV in younger patients is not significantly different from older patients, suggesting a similar role for HPV in all age groups. In the present study, the higher frequency of HPV in patients over 40 years of age could reflect the development of OSCC after a long period of persistence of HPV, or perhaps an age-linked accumulation of risk of acquiring HPV (unrelated to any OSCC risk).

Recent data by Koutsky *et al.* 2002, suggests that in immunocompromised patients a defective immune response may be an additional important factor that leads to progression from potentially malignant to OSCC. The present results indicate that HPV was detected in 78.1% and 56.7% of OSCC patients from the UK and KSA respectively with no indication of immunodeficiency. In addition, HPV was detected among patients who had received no chemo/radiotherapy, hence, it would seem that immunosuppression is not a major influence upon the oral acquisition of HPV in the present samples.

In the present study, the prevalence of HPV in KSA-OSCC samples was found to be 56.7%. A number of features within the KSA group suggest that HPV is not of importance in the development of these tumours. HPV was detected in (71.4%) of females and in (52.2%) of males, yet OSCC is more common in males than females in KSA (Akhtar *et al.*, 2003). In addition, HPV was detected in patients aged over 60 years, yet the mean age of OSCC in KSA residents is 47.7 (Akhtar *et al.*, 2003). Taking into consideration age and gender, the present evidence do not suggest that HPV may be playing a significant role in the development of OSCC in KSA, although the data from the present study does not permit a formal statistical testing of this hypothesis since there was no available KSA normal control samples to compare with. It seems to be that tobacco habits are probably a more likely cause of OSCC than HPV among patients from the KSA. Oral squamous cell carcinoma is the third most common cause of malignancy in KSA after lymphoma and leukaemia (Al-Balawi and Nwoku, 2002), and it has been suggested that smokeless tobacco and a Shamma habit are strongly related

to the type of cancer in KSA. In the South Western region (Gazan) of Saudi Arabia, where the use of White Shamma is very common, oral cancer accounts for up to 20% of all cancers diagnosed in local people (Ibrahim *et al.*, 1986). While the exact geographic origin of the present group of patients is not clear, it is likely that many of the present group of KSA patients were residents of the South West of this country, as the Saudi samples were obtained from referral centers in the capital (Riyadh) known to manage oral cancer cases referred from the South West region of the KSA (Al-Balawi and Nwoku, 2002; Akhtar *et al.*, 2003).

HPV was commonly associated with OSCC of the buccal mucosa and lateral border of tongue in patients from both KSA and the UK. However, there were no specific trends in the location of HPV-associated tumours. The present observation of the tongue being a site of HPV-associated OSCC is in agreement with previous studies (Ha and Califano, 2004), but there is a need to explore this in more detail. It is known that the oropharynx is a more likely site of detection of HPV in persons without OSCC (Chen *et al.*, 2005b), thus explaining the possible association of HPV with tonsillar SCC (Syrjanen, 2004), but there are no studies of the intra-oral site specificity of HPV in individuals without OSCC.

7.1.2 HPV and potentially malignant oral diseases

In the present study, HPV was detected in 42.2% of the examined specimens of OED from the UK. The frequency of HPV was high when compared with that of normal control samples (0.0%). The results of the current study are in agreement with those of studies from India (33.8%) (D'Costa *et al.*, 1998) and the UK (33.3%) (Elamin *et al.*, 1998) that utilized

similar PCR methods targeting L1-ORF. The presently observed frequency is however less than that of a study of Japanese patients in which 60% of lesions contained HPV-DNA (Sugiyama *et al.*, 2003).

Oral squamous cell carcinoma has been recognized to follow a genetic multistep progression from normal to malignant disease (Chen *et al.*, 2005a). The most likely clinically and histopathologically detectable lesions to progress to OSCC remains oral epithelial dysplasia (OED), the progression to OSCC usually being predictable based upon the degree of dysplasia (Ha and Califano, 2004; Syrjanen, 2005). Hence, if HPV was to play an aetiological role in the pathogenesis of HNSCC, it should be present in such potentially malignant lesions. Certainly, HPV is present in about 40% of the present group of OED lesions; however, there was no correlation between the frequency of HPV and the degree of dysplasia. This would thus suggest that HPV does not cause dysplasia of oral mucosa, but that HPV carriage may be a secondary event, perhaps to alcohol and tobacco usage. The present study did not attempt to determine if the presence of HPV within OED was associated with later changes to a more severe form of OED, nor transformation to OSCC. Thus, it remains unclear whether HPV causes OED or drives it to malignancy. Previous studies of potentially malignant disease have often failed to indicate whether the lesions were truly dysplastic. However, the results of the present study of known dysplasia suggest that HPV is not of primary aetiological importance in the development of cellular atypia of the oral mucosa.

The results of the detection of HPV in PVL do not elucidate the link between HPV and potentially malignant and malignant oral mucosal

diseases. Previous studies have reported that HPV may be present in 10-100% of the examined lesions (Hansen *et al.*, 1985; Palefsky *et al.*, 1995; Gopalakrishnan *et al.*, 1997; Silverman S Jr and Gorsky, 1997; Batsakis *et al.*, 1999; Fettig *et al.*, 2000; Femiano *et al.*, 2001; Campisi *et al.*, 2004). In the present study, 58.3% of PVL lesions from the UK contained HPV-DNA. While the outcomes of the present group of patients with PVL are not known, the close similarity in rates of HPV detection in PVL and OED would suggest that HPV is not an important factor in causing or perhaps influencing the behaviour of PVL.

An association between HPV and oral lichen planus (OLP) has been previously reported. In the present study 30% of examined OLP lesions contained HPV-DNA which is in agreement with most (Sand *et al.*, 2000; Giovannelli *et al.*, 2002; Ostwald *et al.*, 2003), but not all, reports (Campisi *et al.*, 2004). The presence of HPV-16 in OLP is unlikely to be the reason that OLP has some malignant potential. Since only <1- 5.3% of OLP lesions are reported to be associated with present or later OSCC (Lo *et al.*, 1998; Mattsson *et al.*, 2002), the high frequency of HPV in OLP (in comparison with its unclear low malignant potential), and the absence of any correlation between HPV presence and degree of dysplasia, all suggest that HPV infection does not account for any malignant transformation of OLP.

7.1.3 HPV in normal control oral mucosa

In the present study, HPV was not detected in any of the normal control samples and is thus, in agreement with some studies (Bouda *et al.*, 2000; Sand *et al.*, 2000; Yang *et al.*, 2004; Kurose *et al.*, 2004; Lin *et al.*, 2005; Hansson *et al.*, 2005), but contradicts other studies whereby HPV has

been detected in the normal control samples (Terai *et al.*, 1999; Sugiyama *et al.*, 2003; Zhang *et al.*, 2004; Shimizu *et al.*, 2004). The general prevalence of HPV in normal oral mucosa has not, as of yet, been clear since the sampling of normal healthy mucosa for relevant findings has not been standardized. In some studies the investigators have examined normal oral mucosa samples adjacent to oral cancer lesions, thus it was not possible to determine whether HPV-DNA was located in the normal tissues or in the adjacent "clinically appeared as lesion-free" epithelium (Maitland *et al.*, 1987; Shindoh *et al.*, 1995; Koppikar *et al.*, 2005). The detection of HPV in normal mucosa may be due to a long latency of HPV infection before clinical/histological change manifested. However, the presence of HPV–DNA in the normal samples might also suggest that most HPV infection does not lead to malignancy.

7.1.4 HPV in HIV-infected individuals

In the present study, the incidence of oral HPV in HIV individuals from UK was relatively high (36.0%) compared with 11.2% in a study of Canadian patients (Coutlee *et al.*, 1997), but higher than (1-4%) in some European studies including Italy, UK, Greece and the Netherlands (Patton *et al.*, 2002). Recently published studies demonstrated that HIV infection increases the frequencies of HPV infection among women (Palefsky and Holly, 2003; Palefsky, 2003). Longitudinal studies have found an increasing incidence of new HPV infections, persistence of HPV-DNA and infection with multiple types of HPV in HIV infected women and men (King *et al.*, 2002; Kreimer *et al.*, 2004). Although there are no reports indicating any strong increase in HNSCC in HIV individuals, if HPV-16 was to be of aetiologically importance

in the development of OSCC, it might be expected that such disease would have been observed in HIV infected individuals, especially as HIV disease is so common worldwide. HIV-infected individuals may also have sexual behaviours which are likely to increase the risk of acquisition of HPV in the mouth (Scully, 2005). However, the potential for OSCC in patients receiving long term HAART does exist as a consequence of their increased longevity of life as patients may maintain social habits likely to give rise to OSCC (e.g. tobacco and alcohol).

7.1.5 HPV in salivary gland neoplasms

The current study found HPV in 5/25 (25.0%) of the examined salivary gland tumours from KSA. This is a high proportion compared with other similar studies (Rommel *et al.*, 1991; Atula *et al.*, 1998; Henley *et al.*, 2004). The failure of the previous studies to detect HPV-DNA may be explained by the nature of HPV in requiring the availability of proliferating mucosal epithelial cells, which is not the case in salivary tissues. As previously mentioned, HPV has no viraemic phase in humans so the infection is not widely disseminated in the body. Thus it would be expected that HPV-associated malignancies would occur site-specifically where the virus enters the body. The detection of HPV in the present group of salivary gland pathologies could reflect the presence of HPV in the overlying oral mucosal tissues near the gland orifices, or perhaps the epithelial lining of the duct and clearly, this needs to be explored more thoroughly.

7.1.6 Relative risk of HPV in OED compared to OSCC

Previous studies have demonstrated that the prevalence of HPV is higher in OED than in OSCC (Sugiyama *et al.*, 2003), possibly suggesting that HPV may be involved in the early stages of carcinogenesis. In contrast to the results of the present study (Table 3.29), the comparison between HPV infection in OED and in OSCC demonstrated that patients with OSCC are approximately 5.3 times more likely to be infected with HPV than patients with OED. In accordance with the observed trend of the meta-analysis described by Miller and Johnstone, the overall results showed that HPV was 2 to 3 times more likely to be detected in potentially malignant lesions and 4.7 times more likely to be detected in OSCC than in normal mucosa (Miller and Johnstone, 2001). This may suggest that HPV does not initiate the malignancy, but the accumulation in the OSCC lesions might act as requisite for the maintainance and progression of proliferation of the malignant state.

7.2 Conclusion of HPV

The present results indicate that there may be an association between HPV and OSCC. HPV is present in oral epithelial dysplasia (OED) and proliferative verrucous leukoplakia (PVL), but there is no evidence of HPV-DNA in the normal oral mucosa. Although the expression of HPV oncogenes was not investigated, the present results suggest that HPV may be present in OSCC and OED and may drive future oncogenesis.

7.3 Herpes viruses in oral mucosal lesions

Human herpes viruses, particularly EBV and HHV-8, are known causes of some malignancies in the head and neck area. EBV is considered as the causative agent of Burkitt's lymphoma, nasopharyngeal carcinoma and opportunistic B cell lymphoma in immunocompromised patients, while HHV-8 gives rise to Kaposi's sarcoma. Few data have been published investigating the role of these herpesviruses and their association with OSCC (Yang *et al.*, 2004).

7. 3.1 Cytomegalovirus

Cytomegalovirus can be present in the oral epithelium and oral fluids of healthy persons (Beyari *et al.*, 2005), indeed the mouth may be a site of residency of this virus. In immunocompromised individuals (e.g. iatrogenic immunosuppression and in particular HIV disease), CMV may gives rise to both oral mucosal and gingival ulceration (Kanas *et al.*, 1987; Schubert *et al.*, 1993; Flaitz *et al.*, 1996; Syrjanen *et al.*, 1999). Cytomegalovirus has the ability to transform a variety of cell lines *in vitro* and thus, may have the ability to be oncogenic, and certainly oral cancer cell lines do contain CMV promoter (Shillitoe and Noonan, 2000). However, there is no evidence that CMV gives rise to oral malignancy in severely immunocompromised individuals such as those with HIV disease (Frezzini *et al.*, 2005), thus, it would seem unlikely that CMV is associated with OSCC or OED.

The present observations of a lack of association of CMV with OSCC are in agreement with previous similar studies. For example, CMV-DNA was not detected in any of 37 examined OSCC samples from Taiwan (Yang *et al.*, 2004). Similarly, the lack of association between CMV and HIV disease

observed in the present study is in agreement with a recent report of HIV-infected individuals from Italy (Ammatuna *et al.*, 2001). While the mouth can be a site of residency of the virus, oral fluids may act as a mean of non-sexual transmission of CMV (Beyari *et al.*, 2005), this virus is unlikely to play any aetiological role in the development of potentially malignant or malignant disease of the mouth.

In the present study, CMV was not found to be associated with salivary gland tumours. This is in agreement with previous such studies of such tumours from Scandinavia (Karja *et al.*, 1997; Atula *et al.*, 1998), and the USA (Laane *et al.*, 2002), and thus, it can be concluded that CMV plays no role in the salivary gland tumourogenesis.

7.3.2 Epstein-Barr virus

The role of Epstein-Barr virus (EBV) in oral carcinogenesis is highly controversial. Studies have reported that the frequency of detection of EBV-DNA or RNA within OSCC to range from 0 to 72% (Horiuchi *et al.*, 1995; Kobayashi *et al.*, 1999; Tsuhako *et al.*, 2000; Cruz *et al.*, 2000; Sand *et al.*, 2002; Tsang *et al.*, 2003; Higa *et al.*, 2003; Iamaroon *et al.*, 2004). The highest frequency of detection of EBV-DNA in OSCC has been observed in Japan (particularly in the south) (Tsuhako *et al.*, 2000), and in contrast, the lowest frequency has been in Scandinavia and the USA (Sand *et al.*, 2002; Goldenberg *et al.*, 2004).

The detection of EBV-DNA in OSCC has been found sometimes to reflect the presence of either EBV-infected saliva or invading lymphocytes (Cruz *et al.*, 1997; Tsuhako *et al.*, 2000), however, the detection of transcripts of EBV oncogenes within tumour cells (e.g. Baw, Hiw, EBER)

(Mao and Smith, 1993; Shimakage *et al.*, 2002; Iamaroon *et al.*, 2004) has suggested that the EBV in OSCC is oncogenically active. Nevertheless, more recent extensive studies of EBV-DNA positive OSCC using a range of molecular biological methods have not detected transcripts of EBV oncogenes EBNER and EBNA-1, nor oncogenic proteins such as a ZEBRA or LMP-1 (Cruz *et al.*, 2000). A recent extensive retrospective investigation of 300 head and neck cancers (exclusive of nasopharyngeal carcinoma) that included 113 OSCC did not detect EBV using quantitative PCR (Goldenberg *et al.*, 2004).

It would thus seem that, while an association between EBV and OSCC may exist in persons from Japan, there is no evidence for such an association in Europeans. There are no data concerning KSA, however, the present results of the individuals with OSCC suggest that EBV is unlikely to play any aetiological role in oral carcinogenesis. The present low prevalence of EBV in OED samples may provide a further evidence for the lack of any carcinogenic role of EBV in OSCC. As in the present study, Epstein-Barr virus has been detected in OED-like lesions (e.g. oral leukoplakia (Shimakage *et al.*, 2002)), but the lack of any statistical association in the present study correlates with the findings of a lack of oncogenic potential for EBV in the oncogenesis of OSCC in Europeans and those from KSA.

Epstein-Barr virus DNA has previously been detected in 26.1% of OLP lesions from patients in Sweden (Sand *et al.*, 2002). In the present study, EBV was not detected in OLP lesions from both UK and KSA. However, in previous studies, EBV in OLP does seem to be predominantly present within the lymphoid element of OLP (Shimakage *et al.*, 2002), it seems unlikely,

particularly when the relevant data concerning OSCC is considered, that EBV plays a role in any malignant potential of OLP.

The detection of EBV-DNA in 50% of PVL lesions is the only reliable finding with respect to this virus and the examined lesions. The link between EBV and PVL is however unclear. PVL is known to contain histological evidence of dysplasia (Hansen *et al.*, 1985), and to be potentially malignant (Silverman S Jr and Gorsky, 1997) and often recurrent. The only oral mucosal lesion with a similar clinical appearance known to be caused by EBV is oral hairy leukoplakia. Oral hairy leukoplakia (OHL) probably reflects an EBV-induced epithelial hyperplasia (Greenspan *et al.*, 1985) and has no known malignant potential (Frezzini *et al.*, 2005) and is almost always associated with immunosuppression (typically HIV disease) (Triantos *et al.*, 1998). The present group of PVL lesions were not distinct to the tongue (a site of EBV binding) (Triantos *et al.*, 1998) and together with the lack of detection of EBV in the present group of patients with HIV disease, and the knowledge that PVL and OHL do not show similar aetiological, histopathological and clinical features, these observations suggest that EBV is not causative of PVL. It may be that EBV is accounting for some of the hyperplasia of PVL, however, as PVL is not distinct to the tongue, this also seems unlikely. Thus, the explanation of the detection of EBV in 50% of the present samples of PVL remains unknown. However, in view of the absence of EBV in OED and OSCC, and the knowledge that immunosuppression-related OHL does not lead to malignant changes. Further exploration of the role of EBV in oral mucosal oncogenesis does not seem warranted.

Epstein-Barr virus may be of an aetiological importance in salivary gland malignancies, the virus being detected in up to 95% of some series of salivary adenolymphoma (Warthin's tumour) (Takezawa *et al.*, 1998), although no association has been observed in other similar studies (Karja *et al.*, 1997; Wolvius *et al.*, 1997; van Heerden *et al.*, 1999; Laane *et al.*, 2002). The association between EBV and pleomorphic adenoma also seems unlikely (Atula *et al.*, 1998; Laane *et al.*, 2002). Epstein-Barr virus may be present in lymphoepithelioma-like carcinoma of the salivary glands, but as this tumour is histopathologically indistinguishable from undifferentiated nasopharyngeal carcinoma (which is associated with EBV), this finding is perhaps not unexpected (Tsai *et al.*, 1996). In the present study, EBV was not found to be associated with salivary gland tumours. Hence, it would seem that EBV plays no role in the pathogenesis of salivary gland tumours, indeed such a link would seem unlikely as there is no increased frequency of salivary gland malignancy (other than non-Hodgkin's lymphoma and Kaposi's sarcoma) in severely immunocompromised patients (Frezzini *et al.*, 2005).

7.3.3 Human herpes virus-8

In the present study, human herpesvirus 8 (HHV-8) was found in (48.0%) of HIV-infected individuals. This was to be expected as HHV-8 can be detected with epithelial cells (Chang *et al.*, 1994; Hengge *et al.*, 2002a) and mucosal ulcers (Di Alberti L. *et al.*, 1997) in HIV-infected individuals. Oral prevalence of HHV-8 has predominantly been associated with HIV disease, however, it is known that this virus can be carried within the mouths of individuals who have neither HIV disease nor Kaposi's sarcoma (acquisition

occurring via non-sexual routes (presumably via saliva)) (Cook *et al.*, 2002b).

Human herpesvirus-8 is the cause of Kaposi's sarcoma, pulmonary effusion lymphoma (PEL) and multicentric Castleman's disease (Hengge *et al.*, 2002b). It has never been found to be associated with an epithelial tumour and the present evidence of a lack of association with any examined potentially malignant or malignant lesions is in agreement with this.

The present data do not suggest that HHV-8 plays a role in the aetiology of non-KS salivary gland tumours. A study of 58 different salivary gland tumours (that included 11 pleomorphic adenomas), HHV-8 was detecccted in one sample of mucosa-associated lymphoid tumour (MALT) in a patient who had Sjogren's syndrome (Klussmann *et al.*, 2000). In addition, HHV-8 was detected in an intra-salivary gland Kaposi's sarcoma in HIV individuals (Castle and Thompson, 2000). Furthermore, in a study of 19 pleomorphic adenomas and 19 malignant salivary gland tumours of persons in Finland, HHV-8 was not detected in any examined lesion (Atula *et al.*, 1998).

The present and previous, lack of an association between HHV-8 and salivary gland malignancy is expected - otherwise it would be likely that salivary gland tumours would be a common feature of HIV disease - which is not the case (Frezzini *et al.*, 2005).

7.4 Human herpes viruses (CMV, EBV and HHV-8) in normal oral mucosa

The present study results detected no HHV-8 among the normal control samples while EBV and CMV were detected in 12.0% and 2.0% of normal samples respectively. The detection of such viruses found to be common within normal mucosa. Hence, the variations of the detection methods, sample size and the nature of the sample would affect the viral proportion within the samples. The complete absence or the low proportion of such viruses of the present study is in accordance with other many studies (Boldogh *et al.*, 1996; Lucht *et al.*, 1998; Ammatuna *et al.*, 2001; Laane *et al.*, 2002; Hao *et al.*, 2003; Yang *et al.*, 2004), which indicate that CMV, EBV and HHV-8 are not commonsal viruses in most of the healthy individuals but the occasional presence of such viruses may indicate a future pathogenic role.

7.5 Conclusion of human herpesviruses

The results of the current study conclude that herpesviruses, Epstein-Barr virus (EBV), cytomegalovirus (CMV) and human herpesvirus-8 (HHV8) play no role in the pathogenesis of potentially malignant and malignant oral diseases, although HHV-8 may be present in HIV-infected individuals and be a cause of later KS.

7.6 Single and double infection of viruses in OSCC

A previous study of oral ulceration unrelated to malignancy indicated that the frequency of carriage of only one virus was far greater than that of the frequencies of carriage of two or more viruses (Lin *et al.*, 2005), thus

perhaps suggesting that, infection with only one virus may suffice to elicit pathology. In the present study, both HPV and HHV-8 were found as single infections in the OSCC samples in 54.7% and 1.6% respectively. In addition, these two viruses were also found as a double infection in 14.0% of the samples, thus suggesting that interactions between HPV and HHV-8 are unlikely to be a common cause of OSCC and OED.

It has been suggested that co-infection of HSV-2 and HPV might enhance HPV-driven oncogenesis. While HSV-2 does not itself cause cervical malignancy. Co-infection of HSV-2 and HPV is common in cervical malignancy (Di Luca *et al.*, 1987; Di Luca *et al.*, 1989), and epidemiological studies indicated that women infected with HSV-2 and HPV are at great risk of developing cervical carcinoma compared to women infected with only one virus (Hildesheim *et al.*, 1991). *In vitro*, studies have demonstrated that HSV-2 transforming fragments (mtr II and mtr III) can act with HPV to transform human cell lines (e.g. fibroblasts and keratinocytes) (Jones, 1995), inducing DNA damage or chromosomal abnormalities in epithelial cells that are latently infected with HPV. In the current study there was no evidence of any interaction between different viruses in the OSCC cases, therefore, suggesting that only one virus was able to cause the malignancy or the sample size was too small to validate the results.

7.7 Conclusions

The results of the present work indicate that an association may exist between HPV and OSCC in persons from the UK. While this may suggest an aetiopathological link between HPV and a malignant potential of the oral mucosa, the lack of association of HPV with the examined potentially malignant oral mucosal disease and OSCC in persons from KSA points away from such an aetiological link. Herpes viruses probably play no role in the development or maintenance of potentially malignant or malignant disease of the oral epithelium. Finally, HPV, CMV, EBV and HHV-8 are unlikely to be of importance in the pathogenesis of tumours derived from salivary gland tissue.

Nevertheless, the continued observations of OSCC arising in persons who neither smoke tobacco nor drink alcohol, the detection of high risk types of HPV that express oncogenes within OSCC and the suggestion that HPV may be particularly associated with OSCC of the posterior tongue, all cannot be ignored, particularly as HPV may be transmitted sexually. It would thus seem important to focus future research upon the frequency of carriage and molecular characteristics of HPV in the posterior tongue and pharynx in sexually active persons to determine if HPV infection truly is an early event in malignant change at this site. Such work will determine whether there is a need to consider vaccine-based prevention of head and neck disease likely to lead to malignant change. Research focused upon possible aetiological associations between known HPV types or herpes viruses with tumours derived from salivary gland tissues should not be pursued.

Appendix 2.1 Genovar DNA extraction protocol

GenoVar diagnostics ltd

NUCLEIC ACID EXTRACTION KIT FOR PARAFFIN EMBEDDED TISSUE

www.genovar.co.uk

GenoVar diagnostics ltd
1030 Heeley Close
Sittingbourne Research Centre
Sittingbourne
KENT
ME9 8HL
United Kingdom

Tel +44 (0) 1795 436688
Fax +44 (0) 1795 436699
Email help@genovar.co.uk

Intended Use:

This kit is designed to extract **total nucleic acid** from formalin-fixed, paraffin embedded tissue. The kit is optimised for extraction from one section up to 20µm in thickness in less than an hour.

Kit Contents:

- 4 bags of 25 reaction tubes containing Extraction Buffer
- 4 tubes containing Powder B.
- 1 bottle containing 20ml of Buffer A. This buffer is sterile so aseptic technique should be used.
- 1 instruction leaflet.

Preparation, Storage and Handling:

- Before use, 4ml of Buffer A should be added to each glass bottle. The resulting solution, Buffer C, should be stored at -20°C. Each 4ml bottle contains enough of Buffer C for 25 extractions.
- Unused glass bottles containing Powder B should be stored at -20°C.

EXTRACTION PROTOCOL:

1. Place the tissue section into a reaction tube on top of the wax layer.
2. Add 150µl of Buffer C to the tube.
3. Centrifuge the tube briefly at low speed to ensure the tissue section is submerged.
4. Heat the tube to 60°C in a heating block, water bath or thermal cycler.
5. Once the wax has melted (usually around 2 minutes at 60°C) mix gently by vortexing.
6. Incubate the sample at 60°C for 30 minutes.
7. Heat the tube to 99°C in a heating block, water bath or thermal cycler and incubate for 5 minutes.
8. Mix the sample vigorously by vortexing and quickly transfer the tube to a non-refrigerated centrifuge (i.e. Benchtop) and spin at full speed for 5 minutes..
9. Place the samples on ice for 5 minutes to ensure the wax layer is fully solidified. This will make the next step easier.
10. Carefully excise or pierce the wax layer using a sterile pipette tip.
11. Remove the liquid phase to a clean fresh tube, taking care not to transfer any resin or cellular debris.
12. Before analysis, centrifuge the nucleic acid to pellet any residual debris, contaminants or resin.
13. Use 0.5 to 5.0µl in PCR (1 or 2µl is usually optimal), or 5 to 10µl for first strand synthesis.

Final buffer (after all additions to the tube)
25mM Tris pH8.3
50mM KCl
2mM MgCl2
0.45% v/v Tween 20
0.45% v/v Nonidet P40
Proteinase K (200 micrograms per ml)

Reference List

Abdulla AK, Mian MY (1996). Lymphoepithelial carcinoma of salivary glands. *Head Neck* 18(6):577-581.

Abdullah AN, Walzman M, Wade A (1993). Treatment of external genital warts comparing cryotherapy (liquid nitrogen) and trichloroacetic acid. *Sex Transm Dis* 20(6):344-345.

Ablashi D, Chatlynne L, Cooper H, Thomas D, Yadav M, Norhanom AW *et al.* (1999). Seroprevalence of human herpesvirus-8 (HHV-8) in countries of Southeast Asia compared to the USA, the Caribbean and Africa. *Br J Cancer* 81(5):893-897.

Ablashi DV, Chatlynne LG, Whitman JE, Jr., Cesarman E (2002). Spectrum of Kaposi's sarcoma-associated herpesvirus, or human herpesvirus 8, diseases. *Clin Microbiol Rev* 15(3):439-464.

ACCP (2004). Planning and Implementing Cervical Cancer Prevention and Control Programs: A Manual for Managers. Alliance for Cervical Cancer Prevention,ACCP; http://www.alliance-cxca.org/; http://www.path.org/files/RH_accp_mfm_p1.pdf.Report No.: Part One, Chapter One.

Akhtar SS, Ijaz AI, Fadl FA, Oyetunji NM (2003). Head and neck cancer in the central region of Saudi Arabia. *Saudi Med J* 24(6):688-690.

Al-Balawi SA, Nwoku AL (2002). Management of oral cancer in a tertiary care hospital. *Saudi Med J* 23(2):156-159.

Amit A, Edwards CL, Athey P, Kaplan AL (2001). Extensive subcutaneous metastases from squamous cell carcinoma of the cervix in patient with HIV. *Int J Gynecol Cancer* 11(1):78-80.

Ammatuna P, Campisi G, Giovannelli L, Giambelluca D, Alaimo C, Mancuso S *et al.* (2001). Presence of Epstein-Barr virus, cytomegalovirus and human papillomavirus in normal oral mucosa of HIV-infected and renal transplant patients. *Oral Dis* 7(1):34-40.

Andreasen JO (1968). Oral lichen planus. 1. A clinical evaluation of 115 cases. *Oral Surg Oral Med Oral Pathol* 25(1):31-42.

Aoki Y, Tosato G (2004). Neoplastic conditions in the context of HIV-1 infection. *Curr HIV Res* 2(4):343-349.

Ascoli V, Signoretti S, Onetti-Muda A, Pescarmona E, la-Rocca C, Nardi F *et al.* (2001). Primary effusion lymphoma in HIV-infected patients with multicentric Castleman's disease. *J Pathol* 193(2):200-209.

Astori G, Beltrame A, Pipan C, Raphenon G, Botta GA (1999). PCR-RFLP-detected human papilloma virus infection in a group of senegalese women attending an STD clinic and identification of a new HPV-68 subtype. *Intervirology* 42(4):221-227.

Atula T, Grenman R, Klemi P, Syrjanen S (1998). Human papillomavirus, Epstein-Barr virus, human herpesvirus 8 and human cytomegalovirus involvement in salivary gland tumours. *Oral Oncol* 34(5):391-395.

Baer R, Bankier AT, Biggin MD, Deininger PL, Farrell PJ, Gibson TJ *et al.* (1984). DNA sequence and expression of the B95-8 Epstein-Barr virus genome. *Nature* 310(5974):207-211.

Baseman JG, Koutsky LA (2005). The epidemiology of human papillomavirus infections. *J Clin Virol* 32 Suppl:16-24.

Batsakis JG, Suarez P, el-Naggar AK (1999). Proliferative verrucous leukoplakia and its related lesions. *Oral Oncol* 35(4):354-359.

Beckmann AM, Daling JR, Sherman KJ, Maden C, Miller BA, Coates RJ *et al.* (1989). Human papillomavirus infection and anal cancer. *Int J Cancer* 43(6):1042-1049.

Bękkers RL, Massuger LF, Bulten J, Melchers WJ (2004). Epidemiological and clinical aspects of human papillomavirus detection in the prevention of cervical cancer. *Rev Med Virol* 14(2):95-105.

Beral V, Newton R (1998). Overview of the epidemiology of immunodeficiency-associated cancers. *J Natl Cancer Inst Monogr*(23):1-6.

Beyari MM, Hodgson TA, Kondowe W, Molyneux EM, Scully C, Porter SR *et al.* (2005). Inter- and intra-person cytomegalovirus infection in Malawian families. *J Med Virol* 75(4):575-582.

Biggar RJ, Rosenberg PS, Cote T (1996). Kaposi's sarcoma and non-Hodgkin's lymphoma following the diagnosis of AIDS. Multistate AIDS/Cancer Match Study Group. *Int J Cancer* 68(6):754-758.

Biggar RJ, Whitby D, Marshall V, Linhares AC, Black F (2000). Human herpesvirus 8 in Brazilian Amerindians: a hyperendemic population with a new subtype. *J Infect Dis* 181(5):1562-1568.

Bigoni B, Dolcetti R, de LL, Carbone A, Boiocchi M, Cassai E *et al.* (1996). Human herpesvirus 8 is present in the lymphoid system of healthy persons and can reactivate in the course of AIDS. *J Infect Dis* 173(3):542-549.

Blackbourn DJ, Ambroziak J, Lennette E, Adams M, Ramachandran B, Levy JA (1997). Infectious human herpesvirus 8 in a healthy North American blood donor. *Lancet* 349(9052):609-611.

Blessing K, McLaren KM, Morris R, Barr BB, Benton EC, Alloub M *et al.* (1990). Detection of human papillomavirus in skin and genital lesions of renal allograft recipients by in situ hybridization. *Histopathology* 16(2):181-185.

Boldogh I, Szaniszlo P, Bresnahan WA, Flaitz CM, Nichols MC, Albrecht T (1996). Kaposi's sarcoma herpesvirus-like DNA sequences in the saliva of individuals infected with human immunodeficiency virus. *Clin Infect Dis* 23(2):406-407.

Borrero JJ, Pujol E, Perez S, Merino D, Montano A, Rodriguez FJ (2002). Plasmablastic lymphoma of the oral cavity and jaws. *AIDS* 16(14):1979-1980.

Bosch FX, de Sanjose (2003). Chapter 1: Human papillomavirus and cervical cancer--burden and assessment of causality. *J Natl Cancer Inst Monogr*(31):3-13.

Bosch FX, Lorincz A, Munoz N, Meijer CJ, Shah KV (2002). The causal relation between human papillomavirus and cervical cancer. *J Clin Pathol* 55(4):244-265.

Bouda M, Gorgoulis VG, Kastrinakis NG, Giannoudis A, Tsoli E, Danassi-Afentaki D *et al.* (2000). "High risk" HPV types are frequently detected in potentially malignant and malignant oral lesions, but not in normal oral mucosa. *Mod Pathol* 13(6):644-653.

Branca M, Costa S, Mariani L, Sesti F, Agarossi A, di CA *et al.* (2004). Assessment of risk factors and human papillomavirus (HPV) related pathogenetic mechanisms of CIN in HIV-positive and HIV-negative women. Study design and baseline data of the HPV-PathogenISS study. *Eur J Gynaecol Oncol* 25(6):689-698.

Branca M, Migliore G, Giuliani ML, Ippolito G, Cappiello G, Garbuglia AR *et al.* (2000). Squamous intraepithelial lesions (SILs) and HPV associated changes in HIV infected women or at risk of HIV. DIANAIDS Cooperative Study Group. *Eur J Gynaecol Oncol* 21(2):155-159.

Braz-Silva PH, Ortega KL, Rezende NP, Nunes FD, Magalhaes MH (2006). Detection of Epstein-Barr virus (EBV) in the oral mucosa of renal transplant patients. *Diagn Cytopathol* 34(1):24-28.

Brenna SM, Syrjanen KJ (2003). Regulation of cell cycles is of key importance in human papillomavirus (HPV)-associated cervical carcinogenesis. *Sao Paulo Med J* 121(3):128-132.

Brennan P, Boffetta P (2004). Mechanistic considerations in the molecular epidemiology of head and neck cancer. *IARC Sci Publ*(157):393-414.

Brentjens MH, Yeung-Yue KA, Lee PC, Tyring SK (2002). Human papillomavirus: a review. *Dermatol Clin* 20(2):315-331.

Buchwald C, Franzmann MB, Jacobsen GK, Juhl BR, Lindeberg H (1997). Carcinomas occurring in papillomas of the nasal septum associated with human papilloma virus (HPV). *Rhinology* 35(2):74-78.

Campione-Piccardo J, Montpetit ML, Gregoire L, Arella M (1991). A highly conserved nucleotide string shared by all genomes of human papillomaviruses. *Virus Genes* 5(4):349-357.

Campisi G, Giovannelli L, Ammatuna P, Capra G, Colella G, Di LC *et al.* (2004). Proliferative verrucous vs conventional leukoplakia: no significantly increased risk of HPV infection. *Oral Oncol* 40(8):835-840.

Carter JJ, Koutsky LA, Hughes JP, Lee SK, Kuypers J, Kiviat N *et al.* (2000). Comparison of human papillomavirus types 16, 18, and 6 capsid antibody responses following incident infection. *J Infect Dis* 181(6):1911-1919.

Carter JJ, Koutsky LA, Wipf GC, Christensen ND, Lee SK, Kuypers J *et al.* (1996). The natural history of human papillomavirus type 16 capsid antibodies among a cohort of university women. *J Infect Dis* 174(5):927-936.

Carvalho AL, Singh B, Spiro RH, Kowalski LP, Shah JP (2004). Cancer of the oral cavity: a comparison between institutions in a developing and a developed nation. *Head Neck* 26(1):31-38.

Castelletto R, Castellsague X, Munoz N, Iscovich J, Chopita N, Jmelnitsky A (1994). Alcohol, tobacco, diet, mate drinking, and esophageal cancer in Argentina. *Cancer Epidemiol Biomarkers Prev* 3(7):557-564.

Castellsague X, Munoz N (2003). Chapter 3: Cofactors in human papillomavirus carcinogenesis--role of parity, oral contraceptives, and tobacco smoking. *J Natl Cancer Inst Monogr*(31):20-28.

Castillo JP, Kowalik TF (2002). Human cytomegalovirus immediate early proteins and cell growth control. *Gene* 290(1-2):19-34.

Castle JT, Thompson LD (2000). Kaposi sarcoma of major salivary gland origin: A clinicopathologic series of six cases. *Cancer* 88(1):15-23.

Castle PE, Schiffman M, Gravitt PE, Kendall H, Fishman S, Dong H *et al.* (2002). Comparisons of HPV DNA detection by MY09/11 PCR methods. *J Med Virol* 68(3):417-423.

Cathomas G (2000). Human herpes virus 8: a new virus discloses its face. *Virchows Arch* 436(3):195-206.

Cathomas G (2003). Kaposi's sarcoma-associated herpesvirus (KSHV)/human herpesvirus 8 (HHV-8) as a tumour virus. *Herpes* 10(3):72-77.

Chan AT, Teo PM, Huang DP (2004). Pathogenesis and treatment of nasopharyngeal carcinoma. *Semin Oncol* 31(6):794-801.

Chan SY, Delius H, Halpern AL, Bernard HU (1995). Analysis of genomic sequences of 95 papillomavirus types: uniting typing, phylogeny, and taxonomy. *J Virol* 69(5):3074-3083.

Chang CH, Chen TH, Hsu RC, Chou PH, Yang JJ, Hwang GY (2005). The prevalence of HPV-18 and variants of E6 gene isolated from cervical cancer patients in Taiwan. *J Clin Virol* 32(1):33-37.

Chang F, Syrjanen S, Nuutinen J, Karja J, Syrjanen K (1990). Detection of human papillomavirus (HPV) DNA in oral squamous cell carcinomas by in situ hybridization and polymerase chain reaction. *Arch Dermatol Res* 282(8):493-497.

Chang KW, Chang CS, Lai KS, Chou MJ, Choo KB (1989). High prevalence of human papillomavirus infection and possible association with betel quid chewing and smoking in oral epidermoid carcinomas in Taiwan. *J Med Virol* 28(1):57-61.

Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM *et al.* (1994). Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science* 266(5192):1865-1869.

Chen R, Aaltonen LM, Vaheri A (2005a). Human papillomavirus type 16 in head and neck carcinogenesis. *Rev Med Virol.*

Chen R, Sehr P, Waterboer T, Leivo I, Pawlita M, Vaheri A *et al.* (2005b). Presence of DNA of human papillomavirus 16 but no other types in tumor-free tonsillar tissue. *J Clin Microbiol* 43(3):1408-1410.

Chow VT, Loh E, Yeo WM, Tan SY, Chan R (2000). Identification of multiple genital HPV types and sequence variants by consensus and nested type-specific PCR coupled with cycle sequencing. *Pathology* 32(3):204-208.

Christensen ND, Kirnbauer R, Schiller JT, Ghim SJ, Schlegel R, Jenson AB *et al.* (1994). Human papillomavirus types 6 and 11 have antigenically distinct strongly immunogenic conformationally dependent neutralizing epitopes. *Virology* 205(1):329-335.

Cinatl J, Jr., Vogel JU, Kotchetkov R, Wilhelm DH (2004). Oncomodulatory signals by regulatory proteins encoded by human cytomegalovirus: a novel role for viral infection in tumor progression. *FEMS Microbiol Rev* 28(1):59-77.

Cioc AM, Allen C, Kalmar JR, Suster S, Baiocchi R, Nuovo GJ (2004). Oral plasmablastic lymphomas in AIDS patients are associated with human herpesvirus 8. *Am J Surg Pathol* 28(1):41-46.

Clarke B, Chetty R (2002). Postmodern cancer: the role of human immunodeficiency virus in uterine cervical cancer. *Mol Pathol* 55(1):19-24.

Clavel C, Rihet S, Masure M, Chypre C, Boulanger JC, Quereux C *et al.* (1998). DNA-EIA to detect high and low risk HPV genotypes in cervical lesions with E6/E7 primer mediated multiplex PCR. *J Clin Pathol* 51(1):38-43.

Clayman GL, Stewart MG, Weber RS, el-Naggar AK, Grimm EA (1994). Human papillomavirus in laryngeal and hypopharyngeal carcinomas. Relationship to survival. *Arch Otolaryngol Head Neck Surg* 120(7):743-748.

Cobbs CS, Harkins L, Samanta M, Gillespie GY, Bharara S, King PH *et al.* (2002). Human cytomegalovirus infection and expression in human malignant glioma. *Cancer Res* 62(12):3347-3350.

Cook RD, Hodgson TA, Molyneux EM, Borgstein E, Porter SR, Teo CG (2002a). Tracking familial transmission of Kaposi's sarcoma-associated herpesvirus using restriction fragment length polymorphism analysis of latent nuclear antigen. *J Virol Methods* 105(2):297-303.

Cook RD, Hodgson TA, Waugh AC, Molyneux EM, Borgstein E, Sherry A *et al.* (2002b). Mixed patterns of transmission of human herpesvirus-8 (Kaposi's sarcoma-associated herpesvirus) in Malawian families. *J Gen Virol* 83(Pt 7):1613-1619.

Cooper K, Evans M, Mount S (2003). Biology and evolution of cervical squamous intraepithelial lesions: a hypothesis with diagnostic prognostic implications. *Adv Anat Pathol* 10(4):200-203.

Coutlee F, Gravitt P, Kornegay J, Hankins C, Richardson H, Lapointe N *et al.* (2002). Use of PGMY primers in L1 consensus PCR improves detection of human papillomavirus DNA in genital samples. *J Clin Microbiol* 40(3):902-907.

Coutlee F, Gravitt P, Richardson H, Hankins C, Franco E, Lapointe N *et al.* (1999). Nonisotopic detection and typing of human papillomavirus DNA in genital samples by the line blot assay. The Canadian Women's HIV study group. *J Clin Microbiol* 37(6):1852-1857.

Coutlee F, Trottier AM, Ghattas G, Leduc R, Toma E, Sanche G *et al.* (1997). Risk factors for oral human papillomavirus in adults infected and not infected with human immunodeficiency virus. *Sex Transm Dis* 24(1):23-31.

Cruz I, van den Brule AJ, Brink AA, Snijders PJ, Walboomers JM, van dW, I *et al.* (2000). No direct role for Epstein-Barr virus in oral carcinogenesis: a study at the DNA, RNA and protein levels. *Int J Cancer* 86(3):356-361.

Cruz I, van den Brule AJ, Steenbergen RD, Snijders PJ, Meijer CJ, Walboomers JM *et al.* (1997). Prevalence of Epstein-Barr virus in oral squamous cell carcinomas, premalignant lesions and normal mucosa--a study using the polymerase chain reaction. *Oral Oncol* 33(3):182-188.

Cruz IB, Snijders PJ, Steenbergen RD, Meijer CJ, Snow GB, Walboomers JM *et al.* (1996). Age-dependence of human papillomavirus DNA presence in oral squamous cell carcinomas. *Eur J Cancer B Oral Oncol* 32B(1):55-62.

Cuschieri KS, Cubie HA, Whitley MW, Seagar AL, Arends MJ, Moore C *et al.* (2004). Multiple high risk HPV infections are common in cervical neoplasia and young women in a cervical screening population. *J Clin Pathol* 57(1):68-72.

D'Costa J, Saranath D, Dedhia P, Sanghvi V, Mehta AR (1998). Detection of HPV-16 genome in human oral cancers and potentially malignant lesions from India. *Oral Oncol* 34(5):413-420.

Daling JR, Sherman KJ, Weiss NS (1986). Risk factors for condyloma acuminatum in women. *Sex Transm Dis* 13(1):16-18.

De Araujo T, Berman B, Weinstein A (2002). Human herpesviruses 6 and 7. *Dermatol Clin* 20(2):301-306.

de Villiers EM (1989). Prevalence of HPV 7 papillomas in the oral mucosa and facial skin of patients with human immunodeficiency virus. *Arch Dermatol* 125(11):1590.

de Villiers EM, Weidauer H, Otto H, zur HH (1985). Papillomavirus DNA in human tongue carcinomas. *Int J Cancer* 36(5):575-578.

Decker LL, Shankar P, Khan G, Freeman RB, Dezube BJ, Lieberman J *et al.* (1996). The Kaposi sarcoma-associated herpesvirus (KSHV) is present as an intact latent genome in KS tissue but replicates in the peripheral blood mononuclear cells of KS patients. *J Exp Med* 184(1):283-288.

Delecluse HJ, Anagnostopoulos I, Dallenbach F, Hummel M, Marafioti T, Schneider U *et al.* (1997). Plasmablastic lymphomas of the oral cavity: a new entity associated with the human immunodeficiency virus infection. *Blood* 89(4):1413-1420.

Devaraj K, Gillison ML, Wu TC (2003). Development of HPV vaccines for HPV-associated head and neck squamous cell carcinoma. *Crit Rev Oral Biol Med* 14(5):345-362.

Dey P (2004). Aneuploidy and malignancy: an unsolved equation. *J Clin Pathol* 57(12):1245-1249.

Di Alberti L., Porter SR, Speight PM, Scully CM, Zakrzewska JM, Williams IG *et al.* (1997). Detection of human herpesvirus-8 DNA in oral ulcer tissues of HIV-infected individuals. *Oral Dis* 3 Suppl 1:S133-S134.

Di Luca D, Costa S, Monini P, Rotola A, Terzano P, Savioli A *et al.* (1989). Search for human papillomavirus, herpes simplex virus and c-myc oncogene in human genital tumors. *Int J Cancer* 43(4):570-577.

Di Luca D, Rotola A, Pilotti S, Monini P, Caselli E, Rilke F *et al.* (1987). Simultaneous presence of herpes simplex and human papilloma virus sequences in human genital tumors. *Int J Cancer* 40(6):763-768.

Dillner J (1999). The serological response to papillomaviruses. *Semin Cancer Biol* 9(6):423-430.

Doniger J, Muralidhar S, Rosenthal LJ (1999). Human cytomegalovirus and human herpesvirus 6 genes that transform and transactivate. *Clin Microbiol Rev* 12(3):367-382.

Ebell MH (2004). Epstein-Barr virus infectious mononucleosis. *Am Fam Physician* 70(7):1279-1287.

Eike A, Buchwald C, Rolighed J, Lindeberg H (1995). Human papillomavirus (HPV) is rarely present in normal oral and nasal mucosa. *Clin Otolaryngol Allied Sci* 20(2):171-173.

Eisen D (1993). The therapy of oral lichen planus. *Crit Rev Oral Biol Med* 4(2):141-158.

Elamin F, Steingrimsdottir H, Wanakulasuriya S, Johnson N, Tavassoli M (1998). Prevalence of human papillomavirus infection in premalignant and malignant lesions of the oral cavity in U.K. subjects: a novel method of detection. *Oral Oncol* 34(3):191-197.

Eliopoulos AG, Young LS (2001). LMP1 structure and signal transduction. *Semin Cancer Biol* 11(6):435-444.

Emery VC (2001). Human herpesviruses 6 and 7 in solid organ transplant recipients. *Clin Infect Dis* 32(9):1357-1360.

Epstein JB, Wan LS, Gorsky M, Zhang L (2003). Oral lichen planus: progress in understanding its malignant potential and the implications for clinical management. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 96(1):32-37.

Evander M, Edlund K, Boden E, Gustafsson A, Jonsson M, Karlsson R *et al.* (1992). Comparison of a one-step and a two-step polymerase chain reaction with degenerate general primers in a population-based study of human papillomavirus infection in young Swedish women. *J Clin Microbiol* 30(4):987-992.

Femiano F, Gombos F, Scully C (2001). Oral proliferative verrucous leukoplakia (PVL); open trial of surgery compared with combined therapy using surgery and methisoprinol in papillomavirus-related PVL. *Int J Oral Maxillofac Surg* 30(4):318-322.

Feoli-Fonseca JC, Oligny LL, Brochu P, Simard P, Falconi S, Yotov WV (2001). Human papillomavirus (HPV) study of 691 pathological specimens from Quebec by PCR-direct sequencing approach. *J Med Virol* 63(4):284-292.

Ferenczy A, Behelak Y, Haber G, Wright TC, Jr., Richart RM (1995). Treating vaginal and external anogenital condylomas with electrosurgery vs CO2 laser ablation. *J Gynecol Surg* 11(1):41-50.

Ferlay J, Parkin DM, Piasani P (2001). Globocan 2000:Cancer incidence and mortality worldwide. Lyon, France.

Fettig A, Pogrel MA, Silverman S Jr, Bramanti TE, Da CM, Regezi JA (2000). Proliferative verrucous leukoplakia of the gingiva. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 90(6):723-730.

Flaitz CM, Nichols CM, Hicks MJ (1996). Herpesviridae-associated persistent mucocutaneous ulcers in acquired immunodeficiency syndrome. A clinicopathologic study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 81(4):433-441.

Folk GS, Abbondanzo SL, Childers EL, Foss RD (2006). Plasmablastic lymphoma: a clinicopathologic correlation. *Ann Diagn Pathol* 10(1):8-12.

Forastiere A, Koch W, Trotti A, Sidransky D (2001). Head and neck cancer. *N Engl J Med* 345(26):1890-1900.

Franco EL, Duarte-Franco E, Ferenczy A (2001). Cervical cancer: epidemiology, prevention and the role of human papillomavirus infection. *CMAJ* 164(7):1017-1025.

Frazer IH, Leonard JH, Schonrock J, Wright RG, Kearsley JH (1993). HPV DNA in oropharyngeal squamous cell cancers: comparison of results from four DNA detection methods. *Pathology* 25(2):138-143.

Fredricks DN, Relman DA (1999). Paraffin removal from tissue sections for digestion and PCR analysis. *Biotechniques* 26(2):198-200.

Frenkel N, Schirmer EC, Wyatt LS, Katsafanas G, Roffman E, Danovich RM *et al.* (1990). Isolation of a new herpesvirus from human CD4+ T cells. *Proc Natl Acad Sci U S A* 87(2):748-752.

Frezzini C, Leao JC, Porter S (2005). Current trends of HIV disease of the mouth. *J Oral Pathol Med* 34(9):513-531.

Frisch M, Biggar RJ (1999). Aetiological parallel between tonsillar and anogenital squamous-cell carcinomas. *Lancet* 354(9188):1442-1443.

Frisch M, Hjalgrim H, Jaeger AB, Biggar RJ (2000). Changing patterns of tonsillar squamous cell carcinoma in the United States. *Cancer Causes Control* 11(6):489-495.

Gaidano G, Cerri M, Capello D, Berra E, Deambrogi C, Rossi D *et al.* (2002). Molecular histogenesis of plasmablastic lymphoma of the oral cavity. *Br J Haematol* 119(3):622-628.

Gandhi MK, Khanna R (2004). Human cytomegalovirus: clinical aspects, immune regulation, and emerging treatments. *Lancet Infect Dis* 4(12):725-738.

Garland SM (2002). Human papillomavirus update with a particular focus on cervical disease. *Pathology* 34(3):213-224.

Gassenmaier A, Hornstein OP (1988). Presence of human papillomavirus DNA in benign and precancerous oral leukoplakias and squamous cell carcinomas. *Dermatologica* 176(5):224-233.

Gazzaniga P, Vercillo R, Gradilone A, Silvestri I, Gandini O, Napolitano M *et al.* (1998). Prevalence of papillomavirus, Epstein-Barr virus, cytomegalovirus, and herpes simplex virus type 2 in urinary bladder cancer. *J Med Virol* 55(4):262-267.

Ghodratnama F, Riggio MP, Wray D (1997). Search for human herpesvirus 6, human cytomegalovirus and varicella zoster virus DNA in recurrent aphthous stomatitis tissue. *J Oral Pathol Med* 26(4):192-197.

Gillison ML (2004). Human papillomavirus-associated head and neck cancer is a distinct epidemiologic, clinical, and molecular entity. *Semin Oncol* 31(6):744-754.

Gillison ML, Koch WM, Capone RB, Spafford M, Westra WH, Wu L *et al.* (2000). Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst* 92(9):709-720.

Gillison ML, Koch WM, Shah KV (1999). Human papillomavirus in head and neck squamous cell carcinoma: are some head and neck cancers a sexually transmitted disease? *Curr Opin Oncol* 11(3):191-199.

Gillison ML, Lowy DR (2004). A causal role for human papillomavirus in head and neck cancer. *Lancet* 363(9420):1488-1489.

Gillison ML, Shah KV (2001). Human papillomavirus-associated head and neck squamous cell carcinoma: mounting evidence for an etiologic role for human papillomavirus in a subset of head and neck cancers. *Curr Opin Oncol* 13(3):183-188.

Giovannelli L, Campisi G, Lama A, Giambalvo O, Osborn J, Margiotta V *et al.* (2002). Human papillomavirus DNA in oral mucosal lesions. *J Infect Dis* 185(6):833-836.

Giroglou T, Sapp M, Lane C, Fligge C, Christensen ND, Streeck RE *et al.* (2001). Immunological analyses of human papillomavirus capsids. *Vaccine* 19(13-14):1783-1793.

Giuliano AR, Harris R, Sedjo RL, Baldwin S, Roe D, Papenfuss MR *et al.* (2002). Incidence, prevalence, and clearance of type-specific human papillomavirus infections: The Young Women's Health Study. *J Infect Dis* 186(4):462-469.

Goedert JJ, Cote TR, Virgo P, Scoppa SM, Kingma DW, Gail MH *et al.* (1998). Spectrum of AIDS-associated malignant disorders. *Lancet* 351(9119):1833-1839.

Goldenberg D, Benoit NE, Begum S, Westra WH, Cohen Y, Koch WM *et al.* (2004). Epstein-Barr virus in head and neck cancer assessed by quantitative polymerase chain reaction. *Laryngoscope* 114(6):1027-1031.

Goldenberg D, Golz A, Netzer A, Rosenblatt E, Rachmiel A, Goldenberg RF *et al.* (2001). Epstein-Barr virus and cancers of the head and neck. *Am J Otolaryngol* 22(3):197-205.

Goncalves MA, Soares EG, Fernandes AP, Fonseca BA, Bettini JS, Simoes RT *et al.* (2004). Langerhans' cell count and HLA class II profile in cervical intraepithelial neoplasia in the presence or absence of HIV infection. *Eur J Obstet Gynecol Reprod Biol* 114(2):221-227.

Gonzalez-Moles MA, Ruiz-Avila I, Gonzalez-Moles S, Martinez I, Ceballos A, Nogales F (1994). Detection of HPV DNA by in situ hybridization in benign, premalignant and malignant lesions of the oral mucosa. *Bull Group Int Rech Sci Stomatol Odontol* 37(3-4):79-85.

Gopalakrishnan R, Weghorst CM, Lehman TA, Calvert RJ, Bijur G, Sabourin CL *et al.* (1997). Mutated and wild-type p53 expression and HPV integration in proliferative verrucous leukoplakia and oral squamous cell carcinoma. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 83(4):471-477.

Gradilone A, Vercillo R, Napolitano M, Cardinali G, Gazzaniga P, Silvestri I *et al.* (1996). Prevalence of human papillomavirus, cytomegalovirus, and Epstein-Barr virus in the cervix of healthy women. *J Med Virol* 50(1):1-4.

Gravitt PE, Burk RD, Lorincz A, Herrero R, Hildesheim A, Sherman ME *et al.* (2003). A comparison between real-time polymerase chain reaction and hybrid capture 2 for human papillomavirus DNA quantitation. *Cancer Epidemiol Biomarkers Prev* 12(6):477-484.

Gravitt PE, Peyton CL, Alessi TQ, Wheeler CM, Coutlee F, Hildesheim A *et al.* (2000). Improved amplification of genital human papillomaviruses. *J Clin Microbiol* 38(1):357-361.

Gravitt PE, Peyton CL, Apple RJ, Wheeler CM (1998). Genotyping of 27 human papillomavirus types by using L1 consensus PCR products by a single-hybridization, reverse line blot detection method. *J Clin Microbiol* 36(10):3020-3027.

Grce M, Husnjak K, Skerlev M, Lipozencic J, Pavelic K (2000). Detection and typing of human papillomaviruses by means of polymerase chain reaction and fragment length polymorphism in male genital lesions. *Anticancer Res* 20(3B):2097-2102.

Greenberg MS (2003). Ulcerative, vesicular, and bullous lesions. In: Burket's oral medicine. Greenberg MS, Glick M, editors. Ontario (Canada): BC Decker, pp. 50-84.

Greenlee RT, Murray T, Bolden S, Wingo PA (2000). Cancer statistics, 2000. *CA Cancer J Clin* 50(1):7-33.

Greenspan D, Canchola AJ, MacPhail LA, Cheikh B, Greenspan JS (2001). Effect of highly active antiretroviral therapy on frequency of oral warts. *Lancet* 357(9266):1411-1412.

Greenspan D, de Villiers EM, Greenspan JS, de Souza YG, zur HH (1988). Unusual HPV types in oral warts in association with HIV infection. *J Oral Pathol* 17(9-10):482-488.

Greenspan JS, Greenspan D, Lennette ET, Abrams DI, Conant MA, Petersen V *et al.* (1985). Replication of Epstein-Barr virus within the epithelial cells of oral "hairy" leukoplakia, an AIDS-associated lesion. *N Engl J Med* 313(25):1564-1571.

Greer RO, Goldman HM (1974). Oral papillomas. Clinicopathologic evaluation and retrospective examination for dyskeratosis in 110 lesions. *Oral Surg Oral Med Oral Pathol* 38(3):435-440.

Greer RO, McDowell JD, Hoernig G (1999). Proliferative verrucous leukoplakia: report of two cases and a discussion of clinicopathology. *J Calif Dent Assoc* 27(4):300-309.

Griffin BE (2000). Epstein-Barr virus (EBV) and human disease: facts, opinions and problems. *Mutat Res* 462(2-3):395-405.

Ha PK, Califano JA (2004). The role of human papillomavirus in oral carcinogenesis. *Crit Rev Oral Biol Med* 15(4):188-196.

Ha PK, Pai SI, Westra WH, Gillison ML, Tong BC, Sidransky D *et al.* (2002). Real-time quantitative PCR demonstrates low prevalence of human papillomavirus type 16 in premalignant and malignant lesions of the oral cavity. *Clin Cancer Res* 8(5):1203-1209.

Hafkamp HC, Manni JJ, Speel EJ (2004). Role of human papillomavirus in the development of head and neck squamous cell carcinomas. *Acta Otolaryngol* 124(4):520-526.

Hagensee ME (2000). Infection with Human Papillomavirus: Update on Epidemiology, Diagnosis, and Treatment. *Curr Infect Dis Rep* 2(1):18-24.

Hansen LS, Olson JA, Silverman S Jr (1985). Proliferative verrucous leukoplakia. A long-term study of thirty patients. *Oral Surg Oral Med Oral Pathol* 60(3):285-298.

Hansson BG, Rosenquist K, Antonsson A, Wennerberg J, Schildt EB, Bladstrom A *et al.* (2005). Strong association between infection with human papillomavirus and oral and oropharyngeal squamous cell carcinoma: a population-based case-control study in southern Sweden. *Acta Otolaryngol* 125(12):1337-1344.

Hao SP, Tsang NM, Chang KP (2003). Screening nasopharyngeal carcinoma by detection of the latent membrane protein 1 (LMP-1) gene with nasopharyngeal swabs. *Cancer* 97(8):1909-1913.

Haraf DJ, Nodzenski E, Brachman D, Mick R, Montag A, Graves D *et al.* (1996). Human papilloma virus and p53 in head and neck cancer: clinical correlates and survival. *Clin Cancer Res* 2(4):755-762.

Harkins L, Volk AL, Samanta M, Mikolaenko I, Britt WJ, Bland KI *et al.* (2002). Specific localisation of human cytomegalovirus nucleic acids and proteins in human colorectal cancer. *Lancet* 360(9345):1557-1563.

Harnish DG, Belland LM, Scheid EE, Rohan TE (1999). Evaluation of human papillomavirus-consensus primers for HPV detection by the polymerase chain reaction. *Mol Cell Probes* 13(1):9-21.

Hart KW, Williams OM, Thelwell N, Fiander AN, Brown T, Borysiewicz LK *et al.* (2001). Novel method for detection, typing, and quantification of human papillomaviruses in clinical samples. *J Clin Microbiol* 39(9):3204-3212.

Hawes SE, Critchlow CW, Faye Niang MA, Diouf MB, Diop A, Toure P *et al.* (2003). Increased risk of high-grade cervical squamous intraepithelial lesions and invasive cervical cancer among African women with human immunodeficiency virus type 1 and 2 infections. *J Infect Dis* 188(4):555-563.

Hemminki K, Dong C, Frisch M (2000). Tonsillar and other upper aerodigestive tract cancers among cervical cancer patients and their husbands. *Eur J Cancer Prev* 9(6):433-437.

Hengge UR, Ruzicka T, Tyring SK, Stuschke M, Roggendorf M, Schwartz RA *et al.* (2002a). Update on Kaposi's sarcoma and other HHV8 associated diseases. Part 1: epidemiology, environmental predispositions, clinical manifestations, and therapy. *Lancet Infect Dis* 2(5):281-292.

Hengge UR, Ruzicka T, Tyring SK, Stuschke M, Roggendorf M, Schwartz RA *et al.* (2002b). Update on Kaposi's sarcoma and other HHV8 associated diseases. Part 2: pathogenesis, Castleman's disease, and pleural effusion lymphoma. *Lancet Infect Dis* 2(6):344-352.

Henley JD, Summerlin DJ, Tomich CE (2004). Condyloma acuminatum and condyloma-like lesions of the oral cavity: a study of 11 cases with an intraductal component. *Histopathology* 44(3):216-221.

Herrero R, Castellsague X, Pawlita M, Lissowska J, Kee F, Balaram P *et al.* (2003). Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. *J Natl Cancer Inst* 95(23):1772-1783.

Herrero R, Hildesheim A, Bratti C, Sherman ME, Hutchinson M, Morales J *et al.* (2000). Population-based study of human papillomavirus infection and cervical neoplasia in rural Costa Rica. *J Natl Cancer Inst* 92(6):464-474.

Heslop HE (2005). Biology and treatment of epstein-barr virus-associated non-hodgkin lymphomas. *Hematology (Am Soc Hematol Educ Program)*:260-266.

Higa M, Kinjo T, Kamiyama K, Chinen K, Iwamasa T, Arasaki A *et al.* (2003). Epstein-Barr virus (EBV)-related oral squamous cell carcinoma in Okinawa, a subtropical island, in southern Japan--simultaneously infected with human papillomavirus (HPV). *Oral Oncol* 39(4):405-414.

Hildesheim A, Mann V, Brinton LA, Szklo M, Reeves WC, Rawls WE (1991). Herpes simplex virus type 2: a possible interaction with human papillomavirus types 16/18 in the development of invasive cervical cancer. *Int J Cancer* 49(3):335-340.

Hille JJ, Webster-Cyriaque J, Palefski JM, Raab-Traub N (2002). Mechanisms of expression of HHV8, EBV and HPV in selected HIV-associated oral lesions. *Oral Dis* 8 Suppl 2:161-168.

Hinchliffe SA, van Velzen D, Korporaal H, Kok PL, Boon ME (1995). Transience of cervical HPV infection in sexually active, young women with normal cervicovaginal cytology. *Br J Cancer* 72(4):943-945.

Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD (1998). Natural history of cervicovaginal papillomavirus infection in young women. *N Engl J Med* 338(7):423-428.

Hoever G, Vogel JU, Lukashenko P, Hofmann WK, Komor M, Doerr HW *et al.* (2005). Impact of persistent cytomegalovirus infection on human neuroblastoma cell gene expression. *Biochem Biophys Res Commun* 326(2):395-401.

Horiuchi K, Mishima K, Ichijima K, Sugimura M, Ishida T, Kirita T (1995). Epstein-Barr virus in the proliferative diseases of squamous epithelium in the oral cavity. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 79(1):57-63.

Huang YQ, Li JJ, Zhang WG, Feiner D, Friedman-Kien AE (1996). Transcription of human herpesvirus-like agent (HHV-8) in Kaposi's sarcoma. *J Clin Invest* 97(12):2803-2806.

Hubbard RA (2003). Human papillomavirus testing methods. *Arch Pathol Lab Med* 127(8):940-945.

Iamaroon A, Khemaleelakul U, Pongsiriwet S, Pintong J (2004). Co-expression of p53 and Ki67 and lack of EBV expression in oral squamous cell carcinoma. *J Oral Pathol Med* 33(1):30-36.

IARC (1995). Human papillomaviruses. Lyon, France: pp. 35-86.

Ibrahim EM, Satti MB, Al Idrissi HY, Higazi MM, Magbool GM, Al QA (1986). Oral cancer in Saudi Arabia: the role of alqat and alshammah. *Cancer Detect Prev* 9(3-4):215-218.

Iftner T, Villa LL (2003). Chapter 12: Human papillomavirus technologies. *J Natl Cancer Inst Monogr*(31):80-88.

Iwatsuki K, Yamamoto T, Tsuji K, Suzuki D, Fujii K, Matsuura H *et al.* (2004). A spectrum of clinical manifestations caused by host immune responses against Epstein-Barr virus infections. *Acta Med Okayama* 58(4):169-180.

Jaber MA, Porter SR, Gilthorpe MS, Bedi R, Scully C (1999). Risk factors for oral epithelial dysplasia--the role of smoking and alcohol. *Oral Oncol* 35(2):151-156.

Jaber MA, Porter SR, Speight P, Eveson JW, Scully C (2003). Oral epithelial dysplasia: clinical characteristics of western European residents. *Oral Oncol* 39(6):589-596.

Jayasooriya PR, Abeyratne S, Ranasinghe AW, Tilakaratne WM (2004). Focal epithelial hyperplasia (Heck's disease): report of two cases with PCR detection of human papillomavirus DNA. *Oral Dis* 10(4):240-243.

Jemal A, Tiwari RC, Murray T, Ghafoor A, Samuels A, Ward E *et al.* (2004). Cancer statistics, 2004. *CA Cancer J Clin* 54(1):8-29.

Jeng KC, Hsu CY, Liu MT, Chung TT, Liu ST (1994). Prevalence of Taiwan variant of Epstein-Barr virus in throat washings from patients with head and neck tumors in Taiwan. *J Clin Microbiol* 32(1):28-31.

Jeon S, Ien-Hoffmann BL, Lambert PF (1995). Integration of human papillomavirus type 16 into the human genome correlates with a selective growth advantage of cells. *J Virol* 69(5):2989-2997.

Jones C (1995). Cervical cancer: is herpes simplex virus type II a cofactor? *Clin Microbiol Rev* 8(4):549-556.

Jontell M, Watts S, Wallstrom M, Levin L, Sloberg K (1990). Human papilloma virus in erosive oral lichen planus. *J Oral Pathol Med* 19(6):273-277.

Jordens JZ, Lanham S, Pickett MA, Amarasekara S, Abeywickrema I, Watt PJ (2000). Amplification with molecular beacon primers and reverse line blotting for the detection and typing of human papillomaviruses. *J Virol Methods* 89(1-2):29-37.

Joyce JG, Tung JS, Przysiecki CT, Cook JC, Lehman ED, Sands JA *et al.* (1999). The L1 major capsid protein of human papillomavirus type 11 recombinant virus-like particles interacts with heparin and cell-surface glycosaminoglycans on human keratinocytes. *J Biol Chem* 274(9):5810-5822.

Kado S, Kawamata Y, Shino Y, Kasai T, Kubota K, Iwasaki H *et al.* (2001). Detection of human papillomaviruses in cervical neoplasias using multiple sets of generic polymerase chain reaction primers. *Gynecol Oncol* 81(1):47-52.

Kanas RJ, Jensen JL, Abrams AM, Wuerker RB (1987). Oral mucosal cytomegalovirus as a manifestation of the acquired immune deficiency syndrome. *Oral Surg Oral Med Oral Pathol* 64(2):183-189.

Kano Y, Shiohara T (2000). Current understanding of cytomegalovirus infection in immunocompetent individuals. *J Dermatol Sci* 22(3):196-204.

Karcioglu ZA, Issa TM (1997). Human papilloma virus in neoplastic and non-neoplastic conditions of the external eye. *Br J Ophthalmol* 81(7):595-598.

Karja V, Syrjanen K, Syrjanen S (1997). No Epstein Barr and cytomegalovirus DNA found in salivary gland tumours. *ORL J Otorhinolaryngol Relat Spec* 59(2):97-99.

Kashima HK, Kutcher M, Kessis T, Levin LS, de Villiers EM, Shah K (1990). Human papillomavirus in squamous cell carcinoma, leukoplakia, lichen planus, and clinically normal epithelium of the oral cavity. *Ann Otol Rhinol Laryngol* 99(1):55-61.

Kay P, Meehan K, Williamson AL (2002). The use of nested polymerase chain reaction and restriction fragment length polymorphism for the detection and typing of mucosal human papillomaviruses in samples containing low copy numbers of viral DNA. *J Virol Methods* 105(1):159-170.

King MD, Reznik DA, O'Daniels CM, Larsen NM, Osterholt D, Blumberg HM (2002). Human papillomavirus-associated oral warts among human immunodeficiency virus-seropositive patients in the era of highly active antiretroviral therapy: an emerging infection. *Clin Infect Dis* 34(5):641-648.

Kirnbauer R, Booy F, Cheng N, Lowy DR, Schiller JT (1992). Papillomavirus L1 major capsid protein self-assembles into virus-like particles that are highly immunogenic. *Proc Natl Acad Sci U S A* 89(24):12180-12184.

Kirnbauer R, Hubbert NL, Wheeler CM, Becker TM, Lowy DR, Schiller JT (1994). A virus-like particle enzyme-linked immunosorbent assay detects serum antibodies in a majority of women infected with human papillomavirus type 16. *J Natl Cancer Inst* 86(7):494-499.

Kjaer SK, Chackerian B, van den Brule AJ, Svare EI, Paull G, Walbomers JM *et al.* (2001). High-risk human papillomavirus is sexually transmitted: evidence from a follow-up study of virgins starting sexual activity (intercourse). *Cancer Epidemiol Biomarkers Prev* 10(2):101-106.

Klussmann JP, Muller A, Wagner M, Guntinas-Lichius O, Jungehuelsing M, Sloots T *et al.* (2000). Human herpesvirus type 8 in salivary gland tumors. *J Clin Virol* 16(3):239-246.

Klussmann JP, Weissenborn SJ, Wieland U, Dries V, Eckel HE, Pfister HJ *et al.* (2003). Human papillomavirus-positive tonsillar carcinomas: a different tumor entity? *Med Microbiol Immunol (Berl)* 192(3):129-132.

Klussmann JP, Weissenborn SJ, Wieland U, Dries V, Kolligs J, Jungehuelsing M *et al.* (2001). Prevalence, distribution, and viral load of human papillomavirus 16 DNA in tonsillar carcinomas. *Cancer* 92(11):2875-2884.

Kobayashi I, Shima K, Saito I, Kiyoshima T, Matsuo K, Ozeki S *et al.* (1999). Prevalence of Epstein-Barr virus in oral squamous cell carcinoma. *J Pathol* 189(1):34-39.

Komatsu TL, Rivero ER, Magalhaes MH, Nunes FD (2005). Epstein-Barr virus in oral hairy leukoplakia scrapes: identification by PCR. *Pesqui Odontol Bras* 19(4):317-321.

Konya J, Veress G, Juhasz A, Szarka K, Sapy T, Hernadi Z *et al.* (2000). Additional human papillomavirus types detected by the hybrid capture tube test among samples from women with cytological and colposcopical atypia. *J Clin Microbiol* 38(1):408-411.

Koppikar P, deVilliers EM, Mulherkar R (2005). Identification of human papillomaviruses in tumors of the oral cavity in an Indian community. *Int J Cancer* 113(6):946-950.

Koskinen WJ, Chen RW, Leivo I, Makitie A, Back L, Kontio R *et al.* (2003). Prevalence and physical status of human papillomavirus in squamous cell carcinomas of the head and neck. *Int J Cancer* 107(3):401-406.

Kotsianti A, Costopoulos J, Morgello S, Papadimitriou C (1996). Undifferentiated carcinoma of the parotid gland in a white patient: detection of Epstein-Barr virus by in situ hybridization. *Hum Pathol* 27(1):87-90.

Koutsky LA, Ault KA, Wheeler CM, Brown DR, Barr E, Alvarez FB *et al.* (2002). A controlled trial of a human papillomavirus type 16 vaccine. *N Engl J Med* 347(21):1645-1651.

Kreimer AR, Alberg AJ, Daniel R, Gravitt PE, Viscidi R, Garrett ES *et al.* (2004). Oral human papillomavirus infection in adults is associated with sexual behavior and HIV serostatus. *J Infect Dis* 189(4):686-698.

Kreimer AR, Clifford GM, Boyle P, Franceschi S (2005). Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. *Cancer Epidemiol Biomarkers Prev* 14(2):467-475.

Kulasingam SL, Myers ER (2003). Potential health and economic impact of adding a human papillomavirus vaccine to screening programs. *JAMA* 290(6):781-789.

Kurose K, Terai M, Soedarsono N, Rabello D, Nakajima Y, Burk RD *et al.* (2004). Low prevalence of HPV infection and its natural history in normal oral mucosa among volunteers on Miyako Island, Japan. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 98(1):91-96.

Kutler DI, Wreesmann VB, Goberdhan A, Ben-Porat L, Satagopan J, Ngai I *et al.* (2003). Human papillomavirus DNA and p53 polymorphisms in squamous cell carcinomas from Fanconi anemia patients. *J Natl Cancer Inst* 95(22):1718-1721.

Laane CJ, Murr AH, Mhatre AN, Jones KD, Lalwani AK (2002). Role of Epstein-Barr virus and cytomegalovirus in the etiology of benign parotid tumors. *Head Neck* 24(5):443-450.

Laconi S, Greco M, Pellegrini-Bettoli P, Rais M, Laconi E, Pani P (2001). One-step detection and genotyping of human papillomavirus in cervical samples by reverse hybridization. *Diagn Mol Pathol* 10(3):200-206.

Lakshmi S, Nair SA, Pillai MR (1993). Oral cancer and human papillomaviruses: is there a link? *J Surg Oncol* 52(3):193-196.

Lambropoulos AF, Dimitrakopoulos J, Frangoulides E, Katopodi R, Kotsis A, Karakasis D (1997). Incidence of human papillomavirus 6, 11, 16, 18 and 33 in normal oral mucosa of a Greek population. *Eur J Oral Sci* 105(4):294-297.

Lawton G, Thomas S, Schonrock J, Monsour F, Frazer I (1992). Human papillomaviruses in normal oral mucosa: a comparison of methods for sample collection. *J Oral Pathol Med* 21(6):265-269.

Lazzari CM, Krug LP, Quadros OF, Baldi CB, Bozzetti MC (2004). Human papillomavirus frequency in oral epithelial lesions. *J Oral Pathol Med* 33(5):260-263.

Lee OJ, Kim KW, Lee GK (2006). Epstein-Barr virus and human immunodeficiency virus-negative oral plasmablastic lymphoma. *J Oral Pathol Med* 35(6):382-384.

Leigh J (2000). Oral warts rise dramatically with use of new agents in HIV. *HIV Clin* 12(2):7.

Lewin F, Norell SE, Johansson H, Gustavsson P, Wennerberg J, Biorklund A *et al.* (1998). Smoking tobacco, oral snuff, and alcohol in the etiology of squamous cell carcinoma of the head and neck: a population-based case-referent study in Sweden. *Cancer* 82(7):1367-1375.

Lin F, Zhang K, Quiery AT, Jr., Prichard J, Schuerch C (2004). Plasmablastic lymphoma of the cervical lymph nodes in a human immunodeficiency virus-negative patient: a case report and review of the literature. *Arch Pathol Lab Med* 128(5):581-584.

Lin SS, Chou MY, Ho CC, Kao CT, Tsai CH, Wang L *et al.* (2005). Study of the viral infections and cytokines associated with recurrent aphthous ulceration. *Microbes Infect* 7(4):635-644.

Liu Y, Wang X, Lo AK, Wong YC, Cheung AL, Tsao SW (2002). Latent membrane protein-1 of Epstein-Barr virus inhibits cell growth and induces sensitivity to cisplatin in nasopharyngeal carcinoma cells. *J Med Virol* 66(1):63-69.

Lo ML, Mignogna MD, Favia G, Procaccini M, Testa NF, Bucci E (1998). The possible association between oral lichen planus and oral squamous cell carcinoma: a clinical evaluation on 14 cases and a review of the literature. *Oral Oncol* 34(4):239-246.

Longworth MS, Laimins LA (2004). Pathogenesis of human papillomaviruses in differentiating epithelia. *Microbiol Mol Biol Rev* 68(2):362-372.

Lowy DR, Gillison ML (2003). A new link between Fanconi anemia and human papillomavirus-associated malignancies. *J Natl Cancer Inst* 95(22):1648-1650.

Lucht E, Brytting M, Bjerregaard L, Julander I, Linde A (1998). Shedding of cytomegalovirus and herpesviruses 6, 7, and 8 in saliva of human immunodeficiency virus type 1-infected patients and healthy controls. *Clin Infect Dis* 27(1):137-141.

Maitland NJ, Cox MF, Lynas C, Prime SS, Meanwell CA, Scully C (1987). Detection of human papillomavirus DNA in biopsies of human oral tissue. *Br J Cancer* 56(3):245-250.

Mao EJ, Smith CJ (1993). Detection of Epstein-Barr virus (EBV) DNA by the polymerase chain reaction (PCR) in oral smears from healthy individuals and patients with squamous cell carcinoma. *J Oral Pathol Med* 22(1):12-17.

Mattsson U, Jontell M, Holmstrup P (2002). Oral lichen planus and malignant transformation: is a recall of patients justified? *Crit Rev Oral Biol Med* 13(5):390-396.

Mbopi-Keou FX, Belec L, Teo CG, Scully C, Porter SR (2002). Synergism between HIV and other viruses in the mouth. *Lancet Infect Dis* 2(7):416-424.

McGregor JM, Farthing A, Crook T, Yu CC, Dublin EA, Levison DA *et al.* (1994). Posttransplant skin cancer: a possible role for p53 gene mutation but not for oncogenic human papillomaviruses. *J Am Acad Dermatol* 30(5 Pt 1):701-706.

McKaig RG, Baric RS, Olshan AF (1998). Human papillomavirus and head and neck cancer: epidemiology and molecular biology. *Head Neck* 20(3):250-265.

Melbye M, Cote TR, Kessler L, Gail M, Biggar RJ (1994). High incidence of anal cancer among AIDS patients. The AIDS/Cancer Working Group. *Lancet* 343(8898):636-639.

Melsheimer P, Vinokurova S, Wentzensen N, Bastert G, von Knebel DM (2004). DNA aneuploidy and integration of human papillomavirus type 16 e6/e7 oncogenes in intraepithelial neoplasia and invasive squamous cell carcinoma of the cervix uteri. *Clin Cancer Res* 10(9):3059-3063.

Meyers C, Andreansky SS, Courtney RJ (2003). Replication and interaction of herpes simplex virus and human papillomavirus in differentiating host epithelial tissue. *Virology* 315(1):43-55.

Miller CS, Johnstone BM (2001). Human papillomavirus as a risk factor for oral squamous cell carcinoma: a meta-analysis, 1982-1997. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 91(6):622-635.

Miller CS, White DK (1996). Human papillomavirus expression in oral mucosa, premalignant conditions, and squamous cell carcinoma: a retrospective review of the literature. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 82(1):57-68.

Mocarski ES, Jr. (2002). Immunomodulation by cytomegaloviruses: manipulative strategies beyond evasion. *Trends Microbiol* 10(7):332-339.

Mogensen TH, Paludan SR (2001). Molecular pathways in virus-induced cytokine production. *Microbiol Mol Biol Rev* 65(1):131-150.

Molijn A, Kleter B, Quint W, Doorn LJ (2005). Molecular diagnosis of human papillomavirus (HPV) infections. *J Clin Virol* 32 Suppl:43-51.

Moodley M (2005). Update on pathophysiologic mechanisms of human papillomavirus. *Curr Opin Obstet Gynecol* 17(1):61-64.

Mork J, Lie AK, Glattre E, Hallmans G, Jellum E, Koskela P *et al.* (2001). Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. *N Engl J Med* 344(15):1125-1131.

Moscicki AB, Shiboski S, Broering J, Powell K, Clayton L, Jay N *et al.* (1998). The natural history of human papillomavirus infection as measured by repeated DNA testing in adolescent and young women. *J Pediatr* 132(2):277-284.

Motoyama S, Ladines-Llave CA, Luis VS, Maruo T (2004). The role of human papilloma virus in the molecular biology of cervical carcinogenesis. *Kobe J Med Sci* 50(1-2):9-19.

Munger K (2002). The role of human papillomaviruses in human cancers. *Front Biosci* 7:d641-d649.

Munger K, Baldwin A, Edwards KM, Hayakawa H, Nguyen CL, Owens M *et al.* (2004). Mechanisms of human papillomavirus-induced oncogenesis. *J Virol* 78(21):11451-11460.

Munoz N (2000). Human papillomavirus and cancer: the epidemiological evidence. *J Clin Virol* 19(1-2):1-5.

Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV *et al.* (2003). Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med* 348(6):518-527.

Nador RG, Cesarman E, Chadburn A, Dawson DB, Ansari MQ, Sald J *et al.* (1996). Primary effusion lymphoma: a distinct clinicopathologic entity associated with the Kaposi's sarcoma-associated herpes virus. *Blood* 88(2):645-656.

Nagpal JK, Patnaik S, Das BR (2002). Prevalence of high-risk human papilloma virus types and its association with P53 codon 72 polymorphism in tobacco addicted oral squamous cell carcinoma (OSCC) patients of Eastern India. *Int J Cancer* 97(5):649-653.

Nazarenko IA, Bhatnagar SK, Hohman RJ (1997). A closed tube format for amplification and detection of DNA based on energy transfer. *Nucleic Acids Res* 25(12):2516-2521.

Ostwald C, Rutsatz K, Schweder J, Schmidt W, Gundlach K, Barten M (2003). Human papillomavirus 6/11, 16 and 18 in oral carcinomas and benign oral lesions. *Med Microbiol Immunol (Berl)* 192(3):145-148.

Pagano JS, Blaser M, Buendia MA, Damania B, Khalili K, Raab-Traub N *et al.* (2004). Infectious agents and cancer: criteria for a causal relation. *Semin Cancer Biol* 14(6):453-471.

Palefsky JM (2003). Cervical human papillomavirus infection and cervical intraepithelial neoplasia in women positive for human immunodeficiency virus in the era of highly active antiretroviral therapy. *Curr Opin Oncol* 15(5):382-388.

Palefsky JM, Holly EA (2003). Chapter 6: Immunosuppression and co-infection with HIV. *J Natl Cancer Inst Monogr*(31):41-46.

Palefsky JM, Holly EA, Gonzales J, Berline J, Ahn DK, Greenspan JS (1991). Detection of human papillomavirus DNA in anal intraepithelial neoplasia and anal cancer. *Cancer Res* 51(3):1014-1019.

Palefsky JM, Holly EA, Ralston ML, Jay N (1998). Prevalence and risk factors for human papillomavirus infection of the anal canal in human immunodeficiency virus (HIV)-positive and HIV-negative homosexual men. *J Infect Dis* 177(2):361-367.

Palefsky JM, Silverman S Jr, bdel-Salaam M, Daniels TE, Greenspan JS (1995). Association between proliferative verrucous leukoplakia and infection with human papillomavirus type 16. *J Oral Pathol Med* 24(5):193-197.

Park TC, Kim CJ, Koh YM, Lee KH, Yoon JH, Kim JH *et al.* (2004). Human papillomavirus genotyping by the DNA chip in the cervical neoplasia. *DNA Cell Biol* 23(2):119-125.

Parkin DM (2006). The global health burden of infection-associated cancers in the year 2002. *Int J Cancer* 118(12):3030-3044.

Pass RF (1985). Epidemiology and transmission of cytomegalovirus. *J Infect Dis* 152(2):243-248.

Patton LL, Phelan JA, Ramos-Gomez FJ, Nittayananta W, Shiboski CH, Mbuguye TL (2002). Prevalence and classification of HIV-associated oral lesions. *Oral Dis* 8 Suppl 2:98-109.

Perrons C, Kleter B, Jelley R, Jalal H, Quint W, Tedder R (2002). Detection and genotyping of human papillomavirus DNA by SPF10 and MY09/11 primers in cervical cells taken from women attending a colposcopy clinic. *J Med Virol* 67(2):246-252.

Pignatelli S, Dal MP, Landini MP (2001). gpUL73 (gN) genomic variants of human cytomegalovirus isolates are clustered into four distinct genotypes. *J Gen Virol* 82(Pt 11):2777-2784.

Pignatelli S, Dal MP, Rossini G, Lazzarotto T, Gatto MR, Landini MP (2003). Intrauterine cytomegalovirus infection and glycoprotein N (gN) genotypes. *J Clin Virol* 28(1):38-43.

Pintos J, Franco EL, Black MJ, Bergeron J, Arella M (1999). Human papillomavirus and prognoses of patients with cancers of the upper aerodigestive tract. *Cancer* 85(9):1903-1909.

Poole LJ, Zong JC, Ciufo DM, Alcendor DJ, Cannon JS, Ambinder R *et al.* (1999). Comparison of genetic variability at multiple loci across the genomes of the major subtypes of Kaposi's sarcoma-associated herpesvirus reveals evidence for recombination and for two distinct types of open reading frame K15 alleles at the right-hand end. *J Virol* 73(8):6646-6660.

Porter SR, Diz DP, Kumar N, Stock C, Barrett AW, Scully C (1999). Oral plasmablastic lymphoma in previously undiagnosed HIV disease. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 87(6):730-734.

Praetorius F (1997). HPV-associated diseases of oral mucosa. *Clin Dermatol* 15(3):399-413.

Pretet JL, Dalstein V, Monnier-Benoit S, Delpeut S, Mougin C (2004). High risk HPV load estimated by Hybrid Capture II correlates with HPV16 load measured by real-time PCR in cervical smears of HPV16-infected women. *J Clin Virol* 31(2):140-147.

Prins M, Meyer L, Hessol NA (2005). Sex and the course of HIV infection in the pre- and highly active antiretroviral therapy eras. *AIDS* 19(4):357-370.

Qu W, Jiang G, Cruz Y, Chang CJ, Ho GY, Klein RS *et al.* (1997). PCR detection of human papillomavirus: comparison between MY09/MY11 and GP5+/GP6+ primer systems. *J Clin Microbiol* 35(6):1304-1310.

Raab MS, Albrecht JC, Birkmann A, Yaguboglu S, Lang D, Fleckenstein B *et al.* (1998). The immunogenic glycoprotein gp35-37 of human herpesvirus 8 is encoded by open reading frame K8.1. *J Virol* 72(8):6725-6731.

Rassekh CH, Rady PL, Arany I, Tyring SK, Knudsen S, Calhoun KH *et al.* (1998). Combined Epstein-Barr virus and human papillomavirus infection in nasopharyngeal carcinoma. *Laryngoscope* 108(3):362-367.

Remmerbach TW, Brinckmann UG, Hemprich A, Chekol M, Kuhndel K, Liebert UG (2004). PCR detection of human papillomavirus of the mucosa: comparison between MY09/11 and GP5+/6+ primer sets. *J Clin Virol* 30(4):302-308.

Richardson H, Kelsall G, Tellier P, Voyer H, Abrahamowicz M, Ferenczy A *et al.* (2003). The natural history of type-specific human papillomavirus infections in female university students. *Cancer Epidemiol Biomarkers Prev* 12(6):485-490.

Riethdorf S, Friedrich RE, Ostwald C, Barten M, Gogacz P, Gundlach KK *et al.* (1997). p53 gene mutations and HPV infection in primary head and neck squamous cell carcinomas do not correlate with overall survival: a long-term follow-up study. *J Oral Pathol Med* 26(7):315-321.

Ringstrom E, Peters E, Hasegawa M, Posner M, Liu M, Kelsey KT (2002). Human papillomavirus type 16 and squamous cell carcinoma of the head and neck. *Clin Cancer Res* 8(10):3187-3192.

Rintala M, Grenman S, Puranen M, Syrjanen S (2006). Natural history of oral papillomavirus infections in spouses: a prospective Finnish HPV Family Study. *J Clin Virol* 35(1):89-94.

Ritchie JM, Smith EM, Summersgill KF, Hoffman HT, Wang D, Klussmann JP *et al.* (2003). Human papillomavirus infection as a prognostic factor in carcinomas of the oral cavity and oropharynx. *Int J Cancer* 104(3):336-344.

Rivera H, Nikitakis NG, Castillo S, Siavash H, Papadimitriou JC, Sauk JJ (2003). Histopathological analysis and demonstration of EBV and HIV p-24 antigen but not CMV expression in labial minor salivary glands of HIV patients affected by diffuse infiltrative lymphocytosis syndrome. *J Oral Pathol Med* 32(7):431-437.

Rockley PF, Tyring SK (1995). Interferons alpha, beta and gamma therapy of anogenital human papillomavirus infections. *Pharmacol Ther* 65(2):265-287.

Roda Husman AM, Walboomers JM, van den Brule AJ, Meijer CJ, Snijders PJ (1995). The use of general primers GP5 and GP6 elongated at their 3' ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. *J Gen Virol* 76 (Pt 4):1057-1062.

Rommel B, de Villiers EM, Durst M, Bartnitzke S, Bullerdiek J (1991). Failure to detect human papillomavirus sequences at the 3p21 rearrangement site in pleomorphic adenomas. *Cancer Genet Cytogenet* 52(2):187-191.

Rosales R, Lopez-Contreras M, Cortes RR (2001). Antibodies against human papillomavirus (HPV) type 16 and 18 E2, E6 and E7 proteins in sera: correlation with presence of papillomavirus DNA. *J Med Virol* 65(4):736-744.

Rousseau P, Stolick M, Morris GM, Ufema J (2004). Case study: Death or damnation--refusing life-prolonging therapy on religious grounds. *Am J Hosp Palliat Care* 21(6):469-473.

Rowshani AT, Bemelman FJ, van Leeuwen EM, van Lier RA, Ten B, I (2005). Clinical and Immunologic Aspects of Cytomegalovirus Infection in Solid Organ Transplant Recipients. *Transplantation* 79(4):381-386.

Sabeti M, Slots J (2004). Herpesviral-bacterial coinfection in periapical pathosis. *J Endod* 30(2):69-72.

Sabeti M, Valles Y, Nowzari H, Simon JH, Kermani-Arab V, Slots J (2003). Cytomegalovirus and Epstein-Barr virus DNA transcription in endodontic symptomatic lesions. *Oral Microbiol Immunol* 18(2):104-108.

Saemundsen AK, Albeck H, Hansen JP, Nielsen NH, Anvret M, Henle W *et al.* (1982). Epstein-Barr virus in nasopharyngeal and salivary gland carcinomas of Greenland Eskimoes. *Br J Cancer* 46(5):721-728.

Salahuddin SZ, Ablashi DV, Markham PD, Josephs SF, Sturzenegger S, Kaplan M *et al.* (1986). Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. *Science* 234(4776):596-601.

Şamanta M, Harkins L, Klemm K, Britt WJ, Cobbs CS (2003). High prevalence of human cytomegalovirus in prostatic intraepithelial neoplasia and prostatic carcinoma. *J Urol* 170(3):998-1002.

Sanclemente G, Gill DK (2002). Human papillomavirus molecular biology and pathogenesis. *J Eur Acad Dermatol Venereol* 16(3):231-240.

Sand L, Jalouli J, Larsson PA, Hirsch JM (2000). Human papilloma viruses in oral lesions. *Anticancer Res* 20(2B):1183-1188.

Sand LP, Jalouli J, Larsson PA, Hirsch JM (2002). Prevalence of Epstein-Barr virus in oral squamous cell carcinoma, oral lichen planus, and normal oral mucosa. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 93(5):586-592.

Santangelo R, D'Ercole S, Graffeo R, Marchetti S, Deli G, Nacci A *et al.* (2004). Bacterial and viral DNA in periodontal disease: a study using multiplex PCR. *New Microbiol* 27(2):133-137.

Sasagawa T, Minemoto Y, Basha W, Yamazaki H, Nakamura M, Yoshimoto H *et al.* (2000). A new PCR-based assay amplifies the E6-E7 genes of most mucosal human papillomaviruses (HPV). *Virus Res* 67(2):127-139.

Saslow D, Runowicz CD, Solomon D, Moscicki AB, Smith RA, Eyre HJ *et al.* (2002). American Cancer Society guideline for the early detection of cervical neoplasia and cancer. *CA Cancer J Clin* 52(6):342-362.

Sato Y, Sugie R, Tsuchiya B, Kameya T, Natori M, Mukai K (2001). Comparison of the DNA extraction methods for polymerase chain reaction amplification from formalin-fixed and paraffin-embedded tissues. *Diagn Mol Pathol* 10(4):265-271.

Saygun I, Yapar M, Ozdemir A, Kubar A, Slots J (2004). Human cytomegalovirus and Epstein-Barr virus type 1 in periodontal abscesses. *Oral Microbiol Immunol* 19(2):83-87.

Schiffman MH (1992a). Validation of hybridization assays: correlation of filter in situ, dot blot and PCR with Southern blot. *IARC Sci Publ*(119):169-179.

Schiffman MH (1992b). Recent progress in defining the epidemiology of human papillomavirus infection and cervical neoplasia. *J Natl Cancer Inst* 84(6):394-398.

Schubert MM, Epstein JB, Lloid ME, Cooney E (1993). Oral infections due to cytomegalovirus in immunocompromised patients. *J Oral Pathol Med* 22(6):268-273.

Schulz TF (2000). Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8): epidemiology and pathogenesis. *J Antimicrob Chemother* 45 Suppl T3:15-27.

Schwartz SM, Daling JR, Doody DR, Wipf GC, Carter JJ, Madeleine MM *et al.* (1998). Oral cancer risk in relation to sexual history and evidence of human papillomavirus infection. *J Natl Cancer Inst* 90(21):1626-1636.

Schwartz SR, Yueh B, McDougall JK, Daling JR, Schwartz SM (2001). Human papillomavirus infection and survival in oral squamous cell cancer: a population-based study. *Otolaryngol Head Neck Surg* 125(1):1-9.

Scully C (2002). Oral squamous cell carcinoma; from an hypothesis about a virus, to concern about possible sexual transmission. *Oral Oncol* 38(3):227-234.

Scully C (2005). Oral cancer; the evidence for sexual transmission. *Br Dent J* 199(4):203-207.

Scully C, el-Kom M (1985). Lichen planus: review and update on pathogenesis. *J Oral Pathol* 14(6):431-458.

Scully C, Porter S (2000). ABC of oral health. Swellings and red, white, and pigmented lesions. *BMJ* 321(7255):225-228.

Severson J, Evans TY, Lee P, Chan T, Arany I, Tyring SK (2001). Human papillomavirus infections: epidemiology, pathogenesis, and therapy. *J Cutan Med Surg* 5(1):43-60.

Sewram V, De SE, Brennan P, Boffetta P (2003). Mate consumption and the risk of squamous cell esophageal cancer in uruguay. *Cancer Epidemiol Biomarkers Prev* 12(6):508-513.

Shillitoe EJ, Noonan S (2000). Strength and specificity of different gene promoters in oral cancer cells. *Oral Oncol* 36(2):214-220.

Shima K, Kobayashi I, Saito I, Kiyoshima T, Matsuo K, Ozeki S *et al.* (2000). Incidence of human papillomavirus 16 and 18 infection and p53 mutation in patients with oral squamous cell carcinoma in Japan. *Br J Oral Maxillofac Surg* 38(5):445-450.

Shimakage M, Horii K, Tempaku A, Kakudo K, Shirasaka T, Sasagawa T (2002). Association of Epstein-Barr virus with oral cancers. *Hum Pathol* 33(6):608-614.

Shimizu M, Adachi A, Zheng S, Matsunaga J, Kusakari Y, Tagami H *et al.* (2004). Detection of various types of human papillomavirus DNA, mainly belonging to the cutaneous-group, more frequently in normal tissue than in squamous cell carcinomas of the lip. *J Dermatol Sci* 36(1):33-39.

Shin DM, Charuruks N, Lippman SM, Lee JJ, Ro JY, Hong WK *et al.* (2001). p53 protein accumulation and genomic instability in head and neck multistep tumorigenesis. *Cancer Epidemiol Biomarkers Prev* 10(6):603-609.

Shindoh M, Chiba I, Yasuda M, Saito T, Funaoka K, Kohgo T *et al.* (1995). Detection of human papillomavirus DNA sequences in oral squamous cell carcinomas and their relation to p53 and proliferating cell nuclear antigen expression. *Cancer* 76(9):1513-1521.

Silverman S Jr, Gorsky M (1997). Proliferative verrucous leukoplakia: a follow-up study of 54 cases. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 84(2):154-157.

Sisk EA, Bradford CR, Jacob A, Yian CH, Staton KM, Tang G *et al.* (2000). Human papillomavirus infection in "young" versus "old" patients with squamous cell carcinoma of the head and neck. *Head Neck* 22(7):649-657.

Slots J (2002). Interactions between herpesviruses and bacteria in human periodontal disease. In: Polymicrobial diseases. Brogden KA, Guthmiller JM, editors. Washington DC: ASM Press, pp. 317-331.

Smith EM, Hoffman HT, Summersgill KS, Kirchner HL, Turek LP, Haugen TH (1998). Human papillomavirus and risk of oral cancer. *Laryngoscope* 108(7):1098-1103.

Smith EM, Ritchie JM, Summersgill KF, Klussmann JP, Lee JH, Wang D *et al.* (2004). Age, sexual behavior and human papillomavirus infection in oral cavity and oropharyngeal cancers. *Int J Cancer* 108(5):766-772.

Smith EM, Summersgill KF, Allen J, Hoffman HT, McCulloch T, Turek LP *et al.* (2000). Human papillomavirus and risk of laryngeal cancer. *Ann Otol Rhinol Laryngol* 109(11):1069-1076.

Sohn YM, Oh MK, Balcarek KB, Cloud GA, Pass RF (1991). Cytomegalovirus infection in sexually active adolescents. *J Infect Dis* 163(3):460-463.

Stoeckle MY (2000). The spectrum of human herpesvirus 6 infection: from roseola infantum to adult disease. *Annu Rev Med* 51:423-430.

Stone KM, Becker TM, Hadgu A, Kraus SJ (1990). Treatment of external genital warts: a randomised clinical trial comparing podophyllin, cryotherapy, and electrodesiccation. *Genitourin Med* 66(1):16-19.

Stoopler ET (2005). Oral herpetic infections (HSV 1-8). *Dent Clin North Am* 49(1):15-29, vii.

Stoopler ET, Greenberg MS (2003). Update on herpesvirus infections. *Dent Clin North Am* 47(3):517-532.

Strauss S, Jordens JZ, Desselberger U, Gray JJ (2000). Single-tube real-time nested polymerase chain reaction for detecting human papillomavirus DNA. *Diagn Mol Pathol* 9(3):151-157.

Sudbo J, Lippman SM, Lee JJ, Mao L, Kildal W, Sudbo A *et al.* (2004). The influence of resection and aneuploidy on mortality in oral leukoplakia. *N Engl J Med* 350(14):1405-1413.

Sugerman PB, Shillitoe EJ (1997). The high risk human papillomaviruses and oral cancer: evidence for and against a causal relationship. *Oral Dis* 3(3):130-147.

Sugiyama M, Bhawal UK, Dohmen T, Ono S, Miyauchi M, Ishikawa T (2003). Detection of human papillomavirus-16 and HPV-18 DNA in normal, dysplastic, and malignant oral epithelium. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 95(5):594-600.

Sun A, Chang JG, Chu CT, Liu BY, Yuan JH, Chiang CP (1998). Preliminary evidence for an association of Epstein-Barr virus with pre-ulcerative oral lesions in patients with recurrent aphthous ulcers or Behcet's disease. *J Oral Pathol Med* 27(4):168-175.

Syrjanen S (2004). HPV infections and tonsillar carcinoma. *J Clin Pathol* 57(5):449-455.

Syrjanen S (2005). Human papillomavirus (HPV) in head and neck cancer. *J Clin Virol* 32 Suppl:59-66.

Syrjanen S (2003). Human papillomavirus infections and oral tumors. *Med Microbiol Immunol (Berl)* 192(3):123-128.

Syrjanen S, Leimola-Virtanen R, Schmidt-Westhausen A, Reichart PA (1999). Oral ulcers in AIDS patients frequently associated with cytomegalovirus (CMV) and Epstein-Barr virus (EBV) infections. *J Oral Pathol Med* 28(5):204-209.

Syrjanen S, Partanen P, Mantyjarvi R, Syrjanen K (1988). Sensitivity of in situ hybridization techniques using biotin- and 35S-labeled human papillomavirus (HPV) DNA probes. *J Virol Methods* 19(3-4):225-238.

Syrjanen SM (1990). Basic concepts and practical applications of recombinant DNA techniques in detection of human papillomavirus (HPV) infection. Review article. *APMIS* 98(2):95-110.

Szentirmay Z, Polus K, Tamas L, Szentkuti G, Kurcsics J, Csernak E *et al.* (2005). Human papillomavirus in head and neck cancer: molecular biology and clinicopathological correlations. *Cancer Metastasis Rev* 24(1):19-34.

Takezawa K, Jackson C, Gnepp DR, King TC (1998). Molecular characterization of Warthin tumor. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 85(5):569-575.

Talacko AA, Teo CG, Griffin BE, Johnson NW (1991). Epstein-Barr virus receptors but not viral DNA are present in normal and malignant oral epithelium. *J Oral Pathol Med* 20(1):20-25.

Talamini R, Franceschi S, Barra S, La Vecchia C (1990). The role of alcohol in oral and pharyngeal cancer in non-smokers, and of tobacco in non-drinkers. *Int J Cancer* 46(3):391-393.

Tan SH, Leong LE, Walker PA, Bernard HU (1994). The human papillomavirus type 16 E2 transcription factor binds with low cooperativity to two flanking sites and represses the E6 promoter through displacement of Sp1 and TFIID. *J Virol* 68(10):6411-6420.

teenyi-Agaba C (1995). Conjunctival squamous-cell carcinoma associated with HIV infection in Kampala, Uganda. *Lancet* 345(8951):695-696.

Terai M, Hashimoto K, Yoda K, Sata T (1999). High prevalence of human papillomaviruses in the normal oral cavity of adults. *Oral Microbiol Immunol* 14(4):201-205.

Triantos D, Boulter AW, Leao JC, Di AL, Porter SR, Scully CM *et al.* (1998). Diversity of naturally occurring Epstein-Barr virus revealed by nucleotide sequence polymorphism in hypervariable domains in the BamHI K and N subgenomic regions. *J Gen Virol* 79 (Pt 11):2809-2817.

Trimble CL, Genkinger JM, Burke AE, Hoffman SC, Helzlsouer KJ, ener-West M *et al.* (2005). Active and passive cigarette smoking and the risk of cervical neoplasia. *Obstet Gynecol* 105(1):174-181.

Tsai CC, Chen CL, Hsu HC (1996). Expression of Epstein-Barr virus in carcinomas of major salivary glands: a strong association with lymphoepithelioma-like carcinoma. *Hum Pathol* 27(3):258-262.

Tsang NM, Chang KP, Lin SY, Hao SP, Tseng CK, Kuo TT *et al.* (2003). Detection of Epstein-Barr virus-derived latent membrane protein-1 gene in various head and neck cancers: is it specific for nasopharyngeal carcinoma? *Laryngoscope* 113(6):1050-1054.

Tsuhako K, Nakazato I, Miyagi J, Iwamasa T, Arasaki A, Hiratsuka H *et al.* (2000). Comparative study of oral squamous cell carcinoma in Okinawa, Southern Japan and Sapporo in Hokkaido, Northern Japan; with special reference to human papillomavirus and Epstein-Barr virus infection. *J Oral Pathol Med* 29(2):70-79.

Tyring SK, Arany I, Stanley MA, Tomai MA, Miller RL, Smith MH *et al.* (1998). A randomized, controlled, molecular study of condylomata acuminata clearance during treatment with imiquimod. *J Infect Dis* 178(2):551-555.

van den Brule AJ, Meijer CJ, Bakels V, Kenemans P, Walboomers JM (1990). Rapid detection of human papillomavirus in cervical scrapes by combined general primer-mediated and type-specific polymerase chain reaction. *J Clin Microbiol* 28(12):2739-2743.

van Heerden WF, Kraft K, Hemmer J, Swart TJ, van Heerden MB, van Rensburg EJ (1999). Warthin's tumour is not an Epstein-Barr virus related disease. *Anticancer Res* 19(4B):2881-2883.

Van Ranst M, Kaplan JB, Burk RD (1992). Phylogenetic classification of human papillomaviruses: correlation with clinical manifestations. *J Gen Virol* 73 (Pt 10):2653-2660.

Vega F, Chang CC, Medeiros LJ, Udden MM, Cho-Vega JH, Lau CC *et al.* (2005). Plasmablastic lymphomas and plasmablastic plasma cell myelomas have nearly identical immunophenotypic profiles. *Mod Pathol* 18(6):806-815.

Venturoli S, Bonvicini F, Cricca M, Gallinella G, Giosa F, Farinazzo F *et al.* (2002). Evaluation of commercial kits for the detection and typing of human papillomavirus in cervical swabs. *J Virol Methods* 105(1):49-56.

Verma S, Nuovo GJ, Porcu P, Baiocchi RA, Crowson AN, Magro CM (2005). Epstein-Barr virus- and human herpesvirus 8-associated primary cutaneous plasmablastic lymphoma in the setting of renal transplantation. *J Cutan Pathol* 32(1):35-39.

Vernon SD, Unger ER, Williams D (2000). Comparison of human papillomavirus detection and typing by cycle sequencing, line blotting, and hybrid capture. *J Clin Microbiol* 38(2):651-655.

Vigliante CE, Quinn PD, Alawi F (2003). Proliferative verrucous leukoplakia: report of a case with characteristic long-term progression. *J Oral Maxillofac Surg* 61(5):626-631.

Volpers C, Schirmacher P, Streeck RE, Sapp M (1994). Assembly of the major and the minor capsid protein of human papillomavirus type 33 into virus-like particles and tubular structures in insect cells. *Virology* 200(2):504-512.

von Doersten PG, Cruz RM, Rasgon BM, Quesenberry CP, Jr., Hilsinger RL, Jr. (1995). Relation between age and head and neck cancer recurrence after surgery: a multivariate analysis. *Otolaryngol Head Neck Surg* 113(3):197-203.

Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV *et al.* (1999). Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol* 189(1):12-19.

Waller J, McCaffery K, Nazroo J, Wardle J (2005). Making sense of information about HPV in cervical screening: a qualitative study. *Br J Cancer* 92(2):265-270.

Walling DM, Ling PD, Gordadze AV, Montes-Walters M, Flaitz CM, Nichols CM (2004). Expression of Epstein-Barr virus latent genes in oral epithelium: determinants of the pathogenesis of oral hairy leukoplakia. *J Infect Dis* 190(2):396-399.

Waterston A, Bower M (2004). Fifty years of multicentric Castleman's disease. *Acta Oncol* 43(8):698-704.

Wen S, Mizugaki Y, Shinozaki F, Takada K (1997a). Epstein-Barr virus (EBV) infection in salivary gland tumors: lytic EBV infection in nonmalignant epithelial cells surrounded by EBV-positive T-lymphoma cells. *Virology* 227(2):484-487.

Wen S, Tsuji T, Li X, Mizugaki Y, Hayatsu Y, Shinozaki F (1997b). Detection and analysis of human papillomavirus 16 and 18 homologous DNA sequences in oral lesions. *Anticancer Res* 17(1A):307-311.

Wheeler CM, Greer CE, Becker TM, Hunt WC, Anderson SM, Manos MM (1996). Short-term fluctuations in the detection of cervical human papillomavirus DNA. *Obstet Gynecol* 88(2):261-268.

White WI, Wilson SD, Bonnez W, Rose RC, Koenig S, Suzich JA (1998). In vitro infection and type-restricted antibody-mediated neutralization of authentic human papillomavirus type 16. *J Virol* 72(2):959-964.

Wilkins K, Turner R, Dolev JC, LeBoit PE, Berger TG, Maurer TA (2006). Cutaneous malignancy and human immunodeficiency virus disease. *J Am Acad Dermatol* 54(2):189-206.

Winer RL, Lee SK, Hughes JP, Adam DE, Kiviat NB, Koutsky LA (2003). Genital human papillomavirus infection: incidence and risk factors in a cohort of female university students. *Am J Epidemiol* 157(3):218-226.

Wolvius EB, Jiwa NM, van d, V, Horstman A, van dW, I (1997). Adenolymphoma and non-Hodgkin's lymphoma of the salivary glands and oral cavity in immunocompetent patients are not associated with latent Epstein-Barr virus. *Oral Oncol* 33(2):119-123.

Woodman CB, Collins S, Winter H, Bailey A, Ellis J, Prior P *et al.* (2001). Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study. *Lancet* 357(9271):1831-1836.

Xi LF, Carter JJ, Galloway DA, Kuypers J, Hughes JP, Lee SK *et al.* (2002). Acquisition and natural history of human papillomavirus type 16 variant infection among a cohort of female university students. *Cancer Epidemiol Biomarkers Prev* 11(4):343-351.

Yang YY, Koh LW, Tsai JH, Tsai CH, Wong EF, Lin SJ *et al.* (2004). Involvement of viral and chemical factors with oral cancer in Taiwan. *Jpn J Clin Oncol* 34(4):176-183.

Yen TL, Murr AH, Rabin J, Mhatre AN, Lalwani AK (2004). Role of cytomegalovirus, Epstein-Barr virus, and human herpes virus-8 in benign lymphoepithelial cysts of the parotid gland. *Laryngoscope* 114(8):1500-1505.

Young LS, Rickinson AB (2004). Epstein-Barr virus: 40 years on. *Nat Rev Cancer* 4(10):757-768.

Zanotti KM, Belinson J (2002). Update on the diagnosis and treatment of human papillomavirus infection. *Cleve Clin J Med* 69(12):948, 951-5, 956.

Zeuss MS, Miller CS, White DK (1991). In situ hybridization analysis of human papillomavirus DNA in oral mucosal lesions. *Oral Surg Oral Med Oral Pathol* 71(6):714-720.

Zhang ZY, Sdek P, Cao J, Chen WT (2004). Human papillomavirus type 16 and 18 DNA in oral squamous cell carcinoma and normal mucosa. *Int J Oral Maxillofac Surg* 33(1):71-74.

Zong J, Ciufo DM, Viscidi R, Alagiozoglou L, Tyring S, Rady P *et al.* (2002). Genotypic analysis at multiple loci across Kaposi's sarcoma herpesvirus (KSHV) DNA molecules: clustering patterns, novel variants and chimerism. *J Clin Virol* 23(3):119-148.

Zong JC, Ciufo DM, Alcendor DJ, Wan X, Nicholas J, Browning PJ *et al.* (1999). High-level variability in the ORF-K1 membrane protein gene at the left end of the Kaposi's sarcoma-associated herpesvirus genome defines four major virus subtypes and multiple variants or clades in different human populations. *J Virol* 73(5):4156-4170.

Zumbach K, Kisseljov F, Sacharova O, Shaichaev G, Semjonova L, Pavlova L *et al.* (2000). Antibodies against oncoproteins E6 and E7 of human papillomavirus types 16 and 18 in cervical-carcinoma patients from Russia. *Int J Cancer* 85(3):313-318.

zur Hausen (1996). Papillomavirus infections--a major cause of human cancers. *Biochim Biophys Acta* 1288(2):F55-F78.

zur Hausen (2002). Papillomaviruses and cancer: from basic studies to clinical application. *Nat Rev Cancer* 2(5):342-350.

zur Hausen (1987). Papillomaviruses in human cancer. *Appl Pathol* 5(1):19-24.

zur Hausen (1991). Human papillomaviruses in the pathogenesis of anogenital cancer. *Virology* 184(1):9-13.

zur Hausen (2002). Papillomaviruses and cancer: from basic studies to clinical application. *Nat Rev Cancer* 2(5):342-350.